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16. ABSTRACT

Carbon tetrachloride (CCl_4) is a haloalkane with a wide range of industrial and chemical applications. Its presence in the atmosphere and in water appears to be of anthropogenic origin. It is readily absorbed through the lung, gastrointestinal tract and skin and, therefore, poses a hazard to human health.

This document is an assessment of the literature available up to March 1983, with the exception of the effect of CCl_4 on stratospheric ozone, to reflect the most recent development in this area. The scientific literature was inventoried, key studies were evaluated and summaries and conclusions were prepared to qualitatively identify the chemical's toxicity and related characteristics. Observed effect levels and dose-response relationships are discussed evaluating the potential toxicity of CCl_4.

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HEALTH ASSESSMENT DOCUMENT FOR CARBON TETRACHLORIDE

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Cincinnati, Ohio 45268

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This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

The Office of Health and Environmental Assessment, in consultation with an Agency work group, has prepared this health assesment to serve as a "source document" for EPA use. Originally the health assessment was developed for use by the Office of Air Quality Planning and Standards; however, at the request of the Agency Work Group on Solvents, the assessment scope was expanded to address multimedia aspects.

In the development of this assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summaries and conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and dose-response relationships are discussed evaluating the potential toxicity of carbon tetrachloride. Unit risk estimates for cancer are calculated to provide a mediaspecific measure of toxicity. This information can then be placed in perspective with observed environmental levels.

AUTHORS, CONTRIBUTORS AND REVIEWERS

The EPA Office of Health and Environmental Assessment (OHEA) is responsible for the preparation of this health assessment document. The OHEA Environmental Criteria and Assessment Office (ECAO/Cin) had overall responsibility for preparation and production of the document (Cynthia Sonich, Principal Author and Project Manager). Each chapter was originally drafted by the authors as listed below. The Health Effects Branch, CSD, ODW provided a draft of the ODW carbon tetrachloride document which was used as the basis for this document.

Authors	**Chapters**
Christopher T. DeRosa, Ph.D. Enviromental Criteria and Assessment Office Cincinnnati, Ohio	6
Richard Hertzberg, Ph.D. Environmental Criteria and Assessment Office Cincinnati, Ohio	5, 14, Appendix
Sheila Rosenthal, Ph.D. Reproductive Effects Assessment Group Washington, DC	10
Cynthia Sonich, M.S. Environmental Criteria and Assessment Office Cincinnati, OH	1, 2, 3, 4, 7, 8, 9, 11, 12, 13, 14, 15
Health Effects Branch Criteria and Standards Division Office of Drinking Water Washington, DC	

The OHEA Carcinogen Assessment Group (CAG) was responsible for reviewing the sections on carcinogenicity. Participating members of the CAG are listed below (principal reviewers for this document are designated by *).

Roy Albert, M.D. (Chairman)*
Elizabeth L. Anderson, Ph.D.
Larry D. Anderson, Ph.D.*
Steven Bayard, Ph.D.*
David L. Bayliss, M.S.
Chao W. Chen, Ph.D.*
Margaret M.L. Chu, Ph.D.
Herman J. Gibb, M.S., M.P.H.
Bernard H. Haberman, D.V.M., M.S.
Charalingayya B. Hiremath, Ph.D.
Robert McGaughy, Ph.D.*
Dharm V. Singh, D.V.M., Ph.D.
Todd W. Thorslund, Sc.D.

The OHEA Reproductive Effects Assessment Group (REAG) was responsible for the authorship and review of sections on mutagenicity. Participating members of the REAG are listed below (principal author of the mutagenicity section is designated by *).

Vicki Vaughan-Dellarco, Ph.D.
John R. Fowle III, Ph.D.
K.S. Lavappa, Ph.D.
Sheila Rosenthal, Ph.D.*
Carol Sakai, Ph.D.
Peter Voytek, Ph.D.

The OHEA Exposure Assessment Group (EAG) was responsible for reviewing the sections on exposure. Participating members of the EAG are listed below.

James Falco, Ph.D.
Gregory Kew, M.S.
Thomas McLaughlin, Ph.D.

The OHEA Environmental Criteria and Assessment Office (ECAO/RTP) provided general peer review of the entire document. Participating members of the ECAO/RTP are listed below.

Lester Grant, Ph.D.
Si Duk Lee, Ph.D.

The following individuals provided peer-review of this draft or earlier drafts of this document:

U.S. Environmental Protection Agency

Dr. Jerry F. Stara
ECAO-Cin
U.S. Environmental Protection Agency

Dr. Michael Dourson
ECAO-Cin
U.S. Environmental Protection Agency

Mr. Steven D. Lutkenhoff
ECAO-Cin
U.S. Environmental Protection Agency

Dr. Debdas Mukerjee
ECAO-Cin
U.S. Environmental Protection Agency

Dr. Herbert L. Wiser
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

External Reviewers

Dr. Tom Clarkson
School of Medicine
University of Rochester
Rochester, New York

Dr. Rolf Hartung
School of Public Health
University of Michigan
Ann Arbor, Michigan

Dr. Magnus Piscator
Karolinska Institute
Stockholm, Sweden

Dr. V.M. Sadagopa Ramanujam
IPA Science Advisor to ECAO-Cin
University of Texas Medical Branch
Galveston, Texas

Dr. James Withey
Food Directorate
Bureau of Food Chemistry
Tunney's Pasture
Ottawa, Canada

In addition, initial review of the document was provided by:

Members of the Agency Work Group on Solvents

Elizabeth L. Anderson	Office of Health and Environmental Assessment
Charles H. Ris	Office of Health and Environmental Assessment
Jean Parker	Office of Health and Environmental Assessment
Mark Greenberg	Office of Health and Environmental Assessment
Cynthia Sonich	Office of Health and Environmental Assessment
Steven D. Lutkenhoff	Office of Health and Environmental Assessment
James A. Stewart	Office of Toxic Substances
Paul Price	Office of Toxic Substances
William Lappenbush	Office of Drinking Water
Hugh Spitzer	Consumer Product Safety Commission
David R. Patrick	Office of Air Quality Planning and Standards
Lois Jacob	Office of General Enforcement

Arnold Edelman	Office of Toxic Integration
Josephine Brecher	Office of Water Regulations and Standards
Mike Ruggiero	Office of Water Regulations and Standards
Jan Jablonski	Office of Solid Waste
Charles Delos	Office of Water Regulations and Standards
Richard Johnson	Office of Pesticide Programs
Priscilla Holtzclaw	Office of Emergency and Remedial Response

EPA Science Advisory Board

The substance of this document was independently peer reviewed in public sessions of the Environmental Health Committee of EPA's Science Advisory Board.

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U.S. Environmental Protection Agency

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1. INTRODUCTION

Carbon tetrachloride (CCl_4), also known as perchloromethane or tetrachloromethane, is a haloalkane with a wide range of industrial and chemical applications. It is a volatile compound yet it is denser than water, thus under certain environmental conditions, such as groundwater contamination or a large spill into a cold body of water, the contamination level may remain relatively constant over time . Its presence in the atmosphere and in water appears to be of anthropogenic origin. As would be expected from its partition coefficients, it is readily absorbed through the lung and gastrointestinal tract and also through the skin.

Toxicological data for nonhuman mammals are extensive and indicate that CCl_4 causes liver and kidney damage primarily but also neurological damage and dermal effects. Case reports on humans document similar effects. The carcinogenicity of CCl_4 has been well-documented with both the International Agency for Research on Cancer and the National Cancer Institute identifying it as an animal carcinogen. It is a suspect human carcinogen.

This document is an assessment of the literature available up to March 1983 with the exception of the effect of CCl_4 on stratospheric ozone.

2. SUMMARY AND CONCLUSIONS

2.1. SUMMARY

Carbon tetrachloride (CCl_4) is a relatively nonpolar compound that is slightly soluble in water, soluble in alcohol and acetone, and miscible in benzene, chloroform and ether. Its density is 1.59 g/mℓ at 4°C which is greater than the density of water. Thus, under favorable conditions, large amounts spilled into water may settle and not volatilize. However, the high vapor pressure of CCl_4 (115.2 mm Hg at 25°C) favors volatilization from water to air.

Carbon tetrachloride is produced commercially from the chlorination of several chemicals such as methane, propane, ethane propylene and carbon disulfide. In 1980, 3.22×10^8 kg were produced in the United States. A much smaller amount of CCl_4 is generated during the production of vinyl chloride and perchloroethylene. The major use of CCl_4 is in the production of chlorofluorocarbons. A reduction in the demand for CCl_4 has resulted in a 3.5% decrease in production over the years 1970-1980. A continued 1.0% decline in production is projected each year through 1985.

Carbon tetrachloride can be detected in the environment using media-specific analytical methods. Levels detected in the environment are generally <0.01 mg/ℓ in water, <0.01 mg/m³ in air, and <0.01 mg/kg in food although higher levels have been detected in urban air and grain fumigated with CCl_4. Food products made from this grain also contain residues of CCl_4. Natural sources of CCl_4 are unknown so that most, if not all CCl_4 present in the environment can be accounted for by anthropogenic activities.

Carbon tetrachloride is extremely stable in the lower atmosphere and troposphere; however, once in the stratosphere, photodissociation is relatively rapid. Its presence in the stratosphere is of concern due to its possible contribution to ozone depletion and subsequent modification of UV-B radiation flux. The extent of this contribution is difficult to estimate for CCl_4 due to several uncertainties in modeling and data as well as uncertainties in emission projections. These estimates have become much more stable in recent years, particularly because of improved understanding of the chemistry. Current estimates of ozone depletion due to CCl_4 alone are 0.3-0.5% derived from discussions and data presented in NAS (1984) and WMO (1982). The steady-state reduction in total column ozone is expected to be 2-5% as estimated by NAS (1984). Increased UV-B radiation studies in the laboratory have shown adverse effects to a variety of terrestrial plant species; for example, in terms of depressed photosynthetic activity, reduced growth rate, increased somatic mutation rates, and inhibition of seed germination. In laboratory studies, some aquatic organisms have also been shown to be adversely affected. Extension of these laboratory findings to the natural environment is being conducted including the adaptability potential to small changes in UV-B flux. Indirect ecosystem effects associated with increases in UV-B radiation flux have been identified to include changes in genetic material and possible alterations in population composition. However, the extent to which these findings apply to the natural environment is still difficult to determine. An increased UV-B flux to human populations is likely to lead to an increase in skin cancers since ~90% of skin cancer (other than melanoma) in the United States is attributed to sunlight in the UV-B region.

When present in water in dilute concentrations, CCl_4 is rapidly lost to the atmosphere by volatilization. However, large quantities of CCl_4 spilled into water may settle and persist, due to its high specific gravity, low water solubility and high chemical stability. In such cases its presence in ambient water or drinking water may present a threat to aquatic ecosystems or human health.

Humans are potentially exposed to CCl_4 through various media. For an adult male, the typical exposure is estimated at 13 μg/day from inhalation, 9 μg/day from fluid intake and 4 μg/day from food intake. Uptake from air appears to be the major source of exposure. For this reason, the fate and transport of CCl_4 has been most extensively studied in air. Information on CCl_4 soil contamination is limited; consequently, its contribution to human exposure is uncertain.

Ecological impacts have been monitored to a limited extent in freshwater and saltwater organisms. Only two freshwater fish and one invertebrate species have been acutely tested; a 96-hour LC_{50} has been determined as low as 27.3 mg/ℓ in the bluegill, Lepomis macrochirus. Chronic toxicity data are not available. However, reported bioconcentration factors are <30 so that tissue residues are insignificant. The only data on saltwater species deals with toxicity following acute exposures. Effects have been identified at levels of 50.0 mg/ℓ. It is noted that the toxic dose for both freshwater and saltwater species can be lower if more sensitive species were tested.

In mammals, CCl_4 is readily absorbed from the lungs and gastrointestinal tract. Absorption also occurs through the skin but at a much slower rate. Following absorption, CCl_4 is distributed to all major organs. Metabolism of the compound occurs primarily in the liver where it is reduced to a trichloromethyl radical and thought to be further metabolized and/or released as a free radical. Excretion of CCl_4 is primarily through the lungs but also occurs in the urine and feces. Quantitative estimates are available only for inhalation absorption efficiency; these range from 30-65%.

Varying degrees of toxicity have been reported in humans and animals following acute, subchronic and chronic exposures via ingestion, inhalation or dermal administration. Such effects can occur systemically as well as locally. For example, cirrhosis of the liver has resulted from inhalation and dermal exposures, whereas lung lesions have resulted from oral ingestion. Animals surviving acute doses have developed a range of effects such as damage to the liver, kidney, lung and central nervous system as well as dermal effects; biochemical alterations were also noted. Animals receiving subchronic and chronic doses have developed kidney and liver damage and, less frequently, damage to the central nervous system. It has been observed that exposure to a higher concentration over a shorter period of time produces a greater effect upon the liver than exposure to a lower concentration over a longer period of time, even though the product of time and concentration is equal in both cases.

In an attempt to verify the purity of CCl_4 used in the testing protocols of the studies in this document, it was observed that CCl_4 of impure or technical grade was not reported; only the use of pure CCl_4 was reported.

Adverse effects recorded for humans following CCl_4 exposure are similar to those in animals. Damage to the liver, kidney, lungs and central nervous system has been documented in various case reports. Biochemical alterations have been identified in case reports and one epidemiological study.

Exposure to CCl_4 did not produce skeletal or functional abnormalities but did result in signs of fetotoxicity. Rats exposed in utero to CCl_4 were noted to have fatty infiltration of the liver from days 1-4 after birth. Carbon tetrachloride has been shown to be transferred to the neonate through mothers' milk. Other adverse reproductive effects include changes in testicular histology eventually resulting in functional infertility.

Carbon tetrachloride has been tested for its mutagenic potential in bacteria, yeast and a mammalian cell line, and for its DNA damaging potential in rat hepatocytes when administered in vivo. Six of the seven point mutation studies in bacteria were negative. The remaining bacterial study was preliminary and only suggestive of a weak mutagenic response. In none of the negative studies was it shown that CCl_4 was activated or metabolized by the exogenous S9 activation system used. Positive mutagenicity and DNA damage results were found in yeast. In contrast, negative DNA damage results were reported, using in vivo unscheduled DNA synthesis as the assay endpoint. DNA binding studies by two laboratories indicate that metabolically activated CCl_4 may interact with DNA.

Carbon tetrachloride has been found to produce carcinogenic or neoplastic responses 1) in the liver of six strains of mice (C3H, B6C3F1, A, Y, C and L); 2) carcinomas in the liver of three strains of rat (Osborne-Mendel, Japanese, Wistar) and hyperplastic nodules in a fourth rat strain (Buffalo) in less than lifetime studies; 3) a small but statistically significant (as compared to pooled controls) hepatocellular carcinoma response in female Osborne-Mendel rats in lifetime experiments; and 4) hepatocellular carcinomas in Syrian golden hamsters.

Case reports of human carcinomas developing years after a history of high CCl_4 exposures are suggestive of but not proof of an association between human carcinogenic risk and exposure to CCl_4. Studies on groups exposed to CCl_4 indicate that further research is necessary.

Estimates of excess human cancer risk from lifetime exposure to CCl_4 have been extrapolated from the cancer studies on animals. Using all models and adjustments as a measure of uncertainty, the upper limit estimates of unit risk following exposure via ingestion are in the range of $5.8x10^{-8}$ to $3.4x10^{-5}$ for exposure to 1 μg/ℓ in drinking water and from $2.3x10^{-7}$ to $1.4x10^{-4}$ for exposure to 1 μg/m³ in air. However, the Agency has selected the linearized multistage model with the dose per body surface area conversion across species and with the exponent k=3 in the adjustment for partial lifetime study. Thus, the upper limit estimates of unit risk are from $3.1x10^{-7}$ to $3.4x10^{-5}$ (geometric mean = $3.7x10^{-6}$) for exposure to 1 μg CCl_4/ℓ drinking water and from $1.2x10^{-6}$ to $1.4x10^{-4}$ (geometric mean = $1.5x10^{-5}$) for exposure to 1 μg CCl_4/m^3 air. No single study was entirely adequate for risk estimation. Thus, the unit risk estimate is based on the geometric mean of the individual unit risk estimates from the four studies considered. Therefore, the Agency's estimate of unit risk for water (lifetime risk at a water concentration of 1 μg/ℓ) is $3.7x10^{-6}$ and for air (lifetime risk at an air concentration of 1 μg/m³) is $1.5x10^{-5}$.

In assessing toxicity, carcinogenicity or any other harmful effect, compounds that react synergistically or antagonistically with CCl_4 must be considered. Identified synergistic substances include ethanol, Kepone, PCB and PBB. Antagonistic effects have been demonstrated with such compounds as chloramphenicol and catechol.

2.2. CONCLUSIONS

Carbon tetrachloride may contribute to the long term, partial destruction of stratospheric ozone. However, several uncertainties in the emissions and modeling make it difficult to quantitatively estimate the extent of ozone depletion attributable to CCl_4.

Carbon tetrachloride causes damage to the liver, lungs, kidneys and central nervous system in humans. These effects are primarily the result of high oral or inhalation exposures. Less severe effects such as biochemical alterations, nausea and headache result from lower exposures or are secondary to the major health hazards attributed to higher exposures. Similar responses have been demonstrated in animals. These animal studies provide useful dose/response data, are well-defined and can identify a causal relationship between the CCl_4 insult and the toxic response. Furthermore, the toxicity from CCl_4 is not only local but also systemic.

Absorption of CCl_4 varies with species. Based upon both human and animal data, absorption coefficients of 40% when route of exposure is via inhalation and 100% when route of exposure is via ingestion are recommended.

The potential exists for embryotoxicity, especially in males. Toxic effects due to CCl_4 have been demonstrated in mammalian fetuses and neonates exposed directly or indirectly via the placenta or mothers' milk, respectively. Teratogenic effects have not been noted following CCl_4 exposure; however, degenerative changes in the testes and subsequent infertility of the offspring have occurred.

The evidence described in this document is insufficient to allow firm conclusions to be made concerning the mutagenic potential of CCl_4. The majority of the mutagenicity studies were negative. The borderline positive results for binding of reactive intermediates to DNA, the positive results in yeast and the highly preliminary evidence for mutagenicity in the one positive bacterial study are insufficient evidence for mutagenicity, but are suggestive of weak genotoxic effects and are an indication that further studies should be done.

Interactions with other chemicals must be considered in assessing the potential health hazards of exposure to CCl_4. Chemicals have been identified that potentiate the effects of CCl_4 as well as those that inhibit the effects of CCl_4.

Carcinogenicity of CCl_4 has been observed in three animal species. The primary lesions are hepatic neoplasms. Cirrhosis, necrosis and cholangiofibrosis have also been found and have been suggested as initial lesions prior to tumorigenesis in liver. Human data on carcinogenicity are restricted to case reports and the preliminary results of one epidemiological study. If the International Agency for Research on Cancer (IARC) criteria were used, this evidence would be classified as sufficient in animals and inadequate in humans for an overall rating of Group 2B. The IARC description for Group 2B ranking is that carbon tetrachloride is probably carcinogenic in humans.

2.2.1. Major Research Needs.

- Experiments in a number of species designed to derive an absorption coefficient (inhalation) or an absorption range (oral) are needed.

- Definitive data on CCl_4-induced carcinogenicity and toxicity in humans including the mechanism of action are needed. While results on short-term exposures are available, they quite often do not contain adequate dose information. Epidemiology studies on occupational groups are warranted as demonstrated in the preliminary study on dry cleaners.

- Long-term studies on animals exposed _in utero_ are needed to assess lifetime effects of such exposures.

- Chronic studies on animals exposed to CCl_4 via drinking water, air or dermal absorption are needed to establish a dose-response relationship of CCl_4's critical effects.

- The toxicity data on the rat and guinea pig are satisfactory. Additional data on mice are needed since, at present, dose/response information is inadequate. Studies on other species would also be useful.

- Toxic effects of chronic exposure on freshwater and saltwater organisms need to be documented.

- Assessment of the overall ecological impact is sparse. Information on soil and air, particularly the stratosphere (levels, fate and transport processes, relative source contribution), is needed, as well as bioaccumulation/bioconcentration in shellfish.

- Additional research to better define the genotoxic potential of CCl_4 is needed.

3. CHEMICAL AND PHYSICAL PROPERTIES/ANALYTICAL METHODOLOGY

3.1. CHEMICAL AND PHYSICAL PROPERTIES

Carbon tetrachloride (CCl_4) is a clear, colorless, nonflammable liquid with a characteristic odor (Windholz, 1976). It is a relatively nonpolar compound that is slightly soluble in water (0.8 g/ℓ at 25°C) (Johns, 1976), soluble in alcohol and acetone, and miscible in benzene, chloroform and ether (Weast, 1978).

Carbon tetrachloride may be quite stable under certain environmental conditions. An estimated 70,000 years are required for half of a given quantity of CCl_4 to decompose in water (Johns, 1976). This decomposition rate is considerably accelerated in the presence of metals such as iron (Pearson and McConnell, 1975). However, hydrolytic decomposition as a means of removal from water appears to be insignificant compared to evaporation, since the properties (Table 3-1) of CCl_4 favor volatilization of the compound from water to air. Carbon tetrachloride has a high vapor pressure of 115.2 mm Hg at 25°C (Johns, 1976). The air/water partition coefficient of CCl_4 at 20°C is 1.1 by volume and ~1000 by weight (Johns, 1976). The rapid vaporization predicted from these properties has been confirmed by Dilling et al. (1975), who reported a CCl_4 evaporation half-life of 29 minutes from a dilute aqueous solution at 25°C.

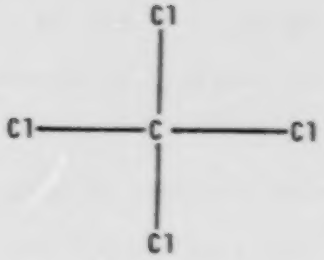
The density of CCl_4 is 1.59 g/mℓ at 4°C (Weast, 1978). Because its density is greater than the density of water, CCl_4 from large spills in water may settle before being totally dispersed, emulsified or volatilized.

Volatilization is the major transport process for removal of CCl_4 from aquatic systems. Once in the troposphere, CCl_4 remains stable; it exhibits an extremely slow rate of reaction with hydroxyl radicals present

TABLE 3-1

Physical and Chemical Properties of Carbon Tetrachloride

A. Structure



$$\begin{array}{ccc} & Cl & \\ & | & \\ Cl - & C & - Cl \\ & | & \\ & Cl & \end{array}$$

B. Synonyms

Tetrachloromethane
Methane tetrachloride
Perchloromethane
Benzinoform
Necatorina

C. Registry Numbers

CAS No. 56-23-5
TSL No. FG 4900000

D. Description

Carbon tetrachloride is a clear, colorless, nonflammable liquid with a charcteristic odor (Windholz, 1976). It is slightly soluble in water, soluble in alcohol and acetone, and miscible in benzene, ether and chloroform (Weast, 1978).

E. Physical Properties

		Reference
1. Molecular weight	153.82	Weast, 1978
2. Melting point	-22.9°C	Weast, 1978
3. Boiling point at 760 torr	76.54°C	Weast, 1978
4. Density in water at 4°C	1.594 g/mℓ	Weast, 1978
at 25°C	1.589 g/mℓ	Windholz, 1976
5. Vapor pressure at 25°C	115.2 mm Hg	Weast, 1978
6. Solubility in water 20°C	785 mg/ℓ	Pearson and McConnell, 1975
7. Log octanol/water partition coefficient	2.64	Neely et al., 1974
8. Conversion factors at 20°C 1 atm.	1 mg/m^3 air = 0.156 ppm in air 1 ppm in air = 6.402 mg/m^3 air	Verschuren, 1977

in the troposphere. The overall atmospheric lifetime of CCl_4 is estimated at 60-100 years. Carbon tetrachloride eventually diffuses into the stratosphere or is carried back to the earth during the precipitation process. Once in the stratosphere, CCl_4 is degraded on exposure to shorter wavelength, higher energy ultraviolet light eventually forming phosgene as the principal product (Federal Register, 1979). See Chapter 5 for an expanded discussion of this issue.

3.2. ANALYTICAL METHODOLOGY

3.2.1. Carbon Tetrachloride in Water.

3.2.1.1. **SAMPLING** -- Grab samples must be collected in glass containers having a total volume in excess of 40 mℓ. The sample bottles should be filled in such a manner that no air bubbles pass through the sample as the bottle is being filled. The bottle should then be sealed so that no bubbles are entrapped in it. The hermetic, nonreactive seal (such as Teflon® tape) should be maintained until the time of analysis.

The samples must be iced or refrigerated from the time of collection until extraction. If the sample is known to contain free or combined chlorine, sodium thiosulfate preservative (10 mg/40 mℓ will suffice for up to 5 mg/ℓ Cl_2) should be added to the empty sample bottles just before shipping to the sampling site. In collection of the sample, the bottle should be filled just to overflowing. After sealing the bottle, the sample should be shaken vigorously for 1 minute. All samples should be analyzed within 14 days of collection (U.S. EPA, 1982); however, samples have been shown to be stable up to 21 days. At 27 days, a 90% mean recovery was measured (Bellar and Lichtenberg, 1981).

3.2.1.2. ANALYSIS

3.2.1.2.1. Ambient Water -- Carbon tetrachloride (and 47 other halogenated organics) in water can be analyzed by a purge and trap method (Method 502.1) described by the U.S. EPA Environmental Monitoring and Support Laboratory (U.S. EPA, 1980b). This method can be used to measure purgeable organics at low concentrations. Purgeable organic compounds are trapped on a Tenax® GC-containing trap at 22°C using a purge gas rate of 40 mℓ/min for 11 minutes. The trapped material is then heated rapidly to 180°C and backflushed with helium at a flow rate of 20-60 mℓ/min for 4 minutes into the gas chromatographic analytical column. The programmable gas chromatograph used is capable of operating at 40°±1°C. The primary analytical column is stainless steel packed with 1% SP-1000 on Carbopack B® (60/80) mesh (8 ft x 0.1 in. i.d.) and is run at a flow rate of 40 mℓ/min. The temperature program sequence begins at 45°C for 3 minutes, increases 8°C/min to 220°C and is then held constant for 15 minutes or until all compounds have eluted. A halogen-specific detector with a sensitivity to 0.10 μg/ℓ and a relative standard deviation of 10% must be used. The optional use of gas chromatography/mass spectroscopy (GC/MS) techniques of comparable accuracy and precision is acceptable.

Carbon tetrachloride can also be detected in water using the headspace gas chromatography method in conjunction with electron capture detection (GC/ECD) described by Dietz and Singley (1979). A 10 ft. by 4 mm (i.d.) glass column was used containing 20% SP-2100/0.1% Carbowax 1500® on 100/120 mesh Supelcoport®. With this method, CCl_4 and other chlorinated hydrocarbons can be detected from 0.1 μg/ℓ to the low mg/ℓ range at ambient temperatures. The headspace method relies on the fact that when a water sample containing organic compounds is sealed in a vial, the organics will equilibrate between the headspace in the vial and the water (Dietz and Singley, 1979). Distribution of the compounds between the two media depends on a number of physical and chemical parameters. With these parameters known, specific measurements can be made on the volatile compounds of interest. Although it is not clearly stated by the authors, accuracy $\geq 98\%$ can be obtained, and assuming a constant and additive term, precision = 0.1.

3.2.1.2.2. Municipal and Industrial Discharges -- The method of analysis is designed to meet the requirements of the National Pollutant Discharge Elimination System (NPDES). In this regard, it presupposes a high expectation of finding the specific compounds of interest in the discharge. It can be utilized to screen samples; however, the user must develop an independent protocol for the verification of identity.

The method of analysis is gas chromatography (GC) and high performance liquid chromatography (HPLC) (Federal Register, 1979). HPLC has been developed considerably in the past few years and can be used to achieve separations and measurements that cannot be performed with state-of-the-art GC. In short, the GC/HPLC method has been developed for the measurement of solvents and other volatile materials using variations of the Bellar purge and trap technique. Semispecific detectors are used to minimize background interferences. A detailed description of the method is provided in the Federal Register (1979).

The sensitivity of this method is usually dependent upon the level of interferences rather than instrumental limitations. The limit of detection for CCl_4 is 0.007 µg/ℓ and represents the sensitivity that can be achieved in wastewaters under optimum operating conditions.

3.2.2. Carbon Tetrachloride in Air.

3.2.2.1. **SAMPLING** -- The most common method applied to ambient air sampling of CCl_4 involves its adsorption onto a suitable medium such as Tenax® GC. Recovery can then be accomplished by thermal desorption and purging with helium into a liquid nitrogen cooled nickel capillary trap (Pellizzari and Bunch, 1979). This method is specific to analysis by GC. Sampling techniques will vary with the analytical methodology.

3.2.2.2. **ANALYSIS** -- Many of the techniques used in analyzing CCl_4 in water apply to air. There are four practical methods to measure air concentrations of CCl_4 [National Academy of Sciences (NAS), 1978]. They are (1) GC/ECD; (2) GC/MS; (3) long path infrared absorption spectroscopy, usually with preconcentration of whole air and then separation of the compounds by gas chromatography (GC/IR); and (4) infrared solar spectroscopy (NAS, 1978).

Gas chromatography/electron capture detection is by far the most widely used method (Lovelock et al., 1973; Lillian et al., 1975; Penkett et al., 1979). The instrumentation needed for this method is readily obtained and relatively inexpensive, costing between $5000 and $10,000 (NAS, 1978). It is sturdy and easy to operate and quite sensitive for CCl_4, which makes it ideal for use on aircraft and ships (Sandalls and Hatton, 1977).

The use of GC/MS has been more recent (Grimsrud and Rasmussen, 1975; Barkley et al., 1980; Dmitriev et al., 1980). Although equally as sensitive as the GC/ECD method, GC/MS has the ability to positively identify compounds by their characteristic mass spectra, whereas GC/ECD must rely upon the somewhat imprecise method of retention times to identify compounds (NAS, 1978). Unfortunately, GC/MS instrumentation is quite expensive, costing $70,000 or more (NAS, 1978). The method employed by Dmitriev et al. (1980)

undertakes the chromatographic separation at room temperature for the first 5 minutes and then at a rising temperature to 150°C at 5°C/minute intervals for a total chromatography time of ~30 minutes, using adsorbants such as Tenax● or Polysorbamide●. Each of the chromatographic fractions can be analyzed by MS. The authors report a sensitivity level of 1 μg/m³ with this method.

Long path infrared absorption spectroscopy with separation by gas chromatography (GC/IR) has the advantage of real-time continuous measurements; however, the disadvantage of poor sensitivity renders this method less desirable (NAS, 1978). Furthermore, the method is expensive, costing $20,000-$100,000, and cannot be used in the field (NAS, 1978). This method is used and reported by Hanst et al. (1975) with a detailed description of sample collection techniques.

Finally, infrared solar spectroscopy has been described by Rasmussen, 1976 and Murcray et al., 1975 (NAS, 1978). This method uses the solar spectrum passing through the atmosphere at large zenith angles to obtain the necessary path length to give sufficient absorption to detect ambient halocarbon levels. Although not in real-time, this method provides continuous data on a remote region of the atmosphere (NAS, 1978), i.e., the stratosphere. However, it is limited to that region alone.

3.2.3. Carbon Tetrachloride in Soil. Little research has been done to detect CCl_4 in soil; however, with concern increasing regarding leachates from landfills and waste disposal sites, the analysis of soil samples for organic compounds has become more important as an indicator of possible groundwater contamination. A recent article by DeLeon et al. (1980) describes a method of analysis for volatile and semivolatile organochloride compounds.

3.2.3.1. **SAMPLING** -- In the method described by DeLeon et al. (1980), samples were taken from vertical borings 30 feet deep using the split-spoon method. The samples were then placed in jars and sealed with Teflon®-lined screw caps. During shipment, they were maintained at 6-10°C. Upon their arrival at the analysis site, they were maintained at -20°C until prepared for analysis.

3.2.3.2. **ANALYSIS** -- The analytical method described by DeLeon et al. (1980) employs a simple extraction procedure using hexane followed by analysis of the extract using temperature programmed GC on high-resolution glass capillary columns with ECD. The electron capture gas chromatographic results are confirmed by mass spectroscopy much like the method used for water sample analysis. Reported sensitivity is at least 10 µg/g. The authors demonstrated this technique to be effective in determining the perimeter and limits of an old chemical waste disposal site and also as an effective method to assess the extent of leaching of chlorocarbons from the waste disposal site into surrounding soils (DeLeon et al., 1980).

3.3. SUMMARY

Carbon tetrachloride is a clear, colorless, nonflammable liquid with a characteristic odor and is slightly soluble in water. Its high vapor pressure favors rapid volatilization from water to air. This characteristic is utilized in most commonly accepted methods of analysis for CCl_4. Gas chromatography, either alone or coupled with mass spectroscopy, is the most widely used detection method. The compound is usually separated from other constitutents in the sample by direct volatilization, extraction or heating, and then analyzed using GC, GC/MS or some other analytical technique. Table 3-2 summarizes the analytical methods discussed in this chapter.

TABLE 3-2

Analytical Methods of Carbon Tetrachloride Detection and Measurement

Medium	Method of Sampling	Analytical Method	Sensitivity	References
Water, ambient	grab sample	purge and trap with GC	0.10 µg/ℓ	U.S. EPA, 1980b
Water, ambient	grab sample	headspace GC with ECD	0.10 µg/ℓ	Dietz and Singley, 1979
Water, municipal and industrial discharges	grab sample	GC/HPLC using purge and trap technique	0.007 µg/ℓ	Federal Register, 1979
Air	adsorption onto appropriate medium followed by desorption and purging	GC/ECD	1 µg/m³	NAS, 1978; Lovelock et al., 1973; Lillian et al., 1979; Penkett et al., 1979; Sandals and Hatton, 1977
Air	adsorption onto appropriate medium followed by desorption and purging	GC/MS	1 µg/m³	Grimsrud and Rasmussen, 1975; Barkley et al., 1980; Dmitriev et al., 1980
Air	adsorption onto appropriate medium followed by desorption and purging	GC/IR	10^{-9} v/v	NAS, 1978; Hanst et al., 1975
Air, stratosphere	in situ measurement, no physical sampling required	IRSS	NS	NAS, 1978; Rasmussen, 1976; Murcray et al., 1975
Soil	split-spoon	GC/ECD confirmed by MS	≤10 µg/ℓ	DeLeon et al., 1980

GC = Gas chromatography; ECD = electron capture detection; MS = mass spectroscopy; HPLC = high performance liquid chromatography; IR = long path infrared absorption spectroscopy; IRSS = infrared solar spectroscopy; NS = not stated

4. PRODUCTION, USE AND ENVIRONMENTAL EXPOSURE LEVELS

4.1. PRODUCTION

Carbon tetrachloride is produced industrially by the chlorination of methane, propane, ethane, propylene or carbon disulfide (Rams et al., 1979). In 1980, 322 million kg were manufactured [U.S. International Trade Commission (USITC), 1981]. Carbon tetrachloride is also produced as a byproduct during the production of compounds such as vinyl chloride and perchloroethylene (Rams et al., 1979).

Production levels have decreased as a result of the rapid decline in its major end product area, manufacture of fluorocarbon aerosols. In 1975 fluorocarbon aerosols had a 50% share of the domestic aerosol market; however, this declined to 20% in 1977 due to the non-essential aerosol ban (Chemical Marketing Reporter, 1981). Fluorocarbons are expected to show some strength as solvents, as a refrigerant and in foam-blowing applications. Growth in these latter areas is expected to counteract the decrease in the manufacture of fluorocarbon aerosols and, therefore, stabilize CCl_4 production in the 1980s.

4.2. USE

Currently the major use of CCl_4 is in the production of chlorofluorocarbons, which are used as refrigerants, foam-blowing agents and solvents. These uses accounted for 95% of the total U.S. consumption in 1980. Carbon tetrachloride is also used in fumigants and has a variety of minor uses (5% of total 1980 consumption), including those as a solvent in metal cleaning and in manufacture of paints and plastics (Rams et al., 1979). It is being replaced in grain fumigation by other registered pesticide products (U.S. EPA, 1980a), and its registration for use in fumigants was under review by U.S. EPA (Federal Register, 1980a). Approximately 12% of the total 1980 production was exported.

4.3. ENVIRONMENTAL EXPOSURE LEVELS

Carbon tetrachloride present in the environment appears to be of anthropogenic origin (Singh et al., 1976). Its presence in surface waters occurs primarily as a result of industrial and agricultural activities, although some may reach surface water through rainfall. Groundwater contamination may be the result of leaching from solid waste sites. This is also a source of soil contamination. Air is the medium wherein the greatest concentrations of CCl_4 can be found in the environment with the major source being industrial emission. Once in the air, CCl_4 can be washed into surface waters and soil through rainfall.

Once in the environment, CCl_4 is relatively stable. Its half-life for hydrolytic breakdown in water at pH 1.0-7.0 is estimated to be 70,000 years, but hydrolysis appears to be accelerated in the presence of metals such as iron and zinc (Johns, 1976). The high stability in water has little practical significance, however, since CCl_4 vaporizes readily to air. The atmospheric lifetime of CCl_4 is estimated to be on the order of 30-100 years (Singh et al., 1976).

The presence of CCl_4 in the environment is of concern for two reasons. As indicated by both animal and human studies, CCl_4 may pose a health problem through direct exposure in the air, water, food and/or soil. However, it has also been postulated that CCl_4 may also contribute to ozone-destroying photochemical reactions in the stratosphere. If this were to occur, it might cause increases in the incidence of human skin cancers and animal cancers, affect terrestrial and aquatic ecosystems, and bring about climatic changes (NAS, 1978, 1982).

Although levels of CCl_4 in the environment are generally in the low ppb range or below (NAS, 1978), CCl_4 may pose a long-term risk because of its possible carcinogenic potential (see Chapter 11). In urban and industrial areas where higher concentrations of CCl_4 in air may occur, other toxic effects may result (e.g., liver and renal damage).

No natural sources of CCl_4 have been reported. The presence of a natural source was suggested because of the large quantity of CCl_4 in the atmosphere and the homogeneity of ambient concentrations in both hemispheres (Lovelock et al., 1973). However, this suggestion has since been challenged, since estimates of cumulative worldwide production and emissions of CCl_4 appear to account for the CCl_4 found in the environment (Singh et al., 1976).

4.3.1. Possible Sources and Levels of Carbon Tetrachloride in Water.

Carbon tetrachloride has been monitored extensively in drinking water and, to a lesser extent, in natural waters. The chemical's concentration in drinking water has been reported as <0.007 mg/ℓ (Symons et al., 1975). Samples of ocean, lake and ground water have generally yielded CCl_4 concentrations in the ng/ℓ (ppt) range. There are some indications that industrial activity may lead to increased CCl_4 concentrations in surface and groundwater. A discussion of these monitoring studies follows.

In the National Organics Reconnaissance Survey (NORS), U.S. EPA found CCl_4 levels of <0.003 mg/ℓ in drinking water in 80 cities (Symons et al., 1975). The more recent National Organics Monitoring Survey (NOMS) of 113 public drinking water systems detected CCl_4 in the range of 0.0024-0.0064 mg/ℓ in 10% of the samples surveyed (U.S. EPA, 1980a). Carbon tetrachloride concentrations in these samples were very low compared to those of chloroform and other organics.

Carbon tetrachloride has been detected in drinking water in Tuscaloosa, Alabama (Bertsch et al., 1975); the District of Columbia (Scheiman et al., 1974); Durham, North Carolina (McKinney et al., 1976); and New Orleans, Louisiana (Dowty et al., 1975). In the District of Columbia, CCl_4 in drinking water was measured at 0.005 mg/ℓ (Scheiman et al., 1974). In New Orleans, higher concentrations of CCl_4 were found in blood plasma than in drinking water, suggesting to the authors the presence of a bioaccumulation mechanism or sources of the compound other than drinking water (Dowty et al., 1975). The former, however, has not been demonstrated. Carbon tetrachloride was also found in drinking water in Germany in the ng/ℓ range (Sonneborn and Bohn, 1977).

Under unusual conditions, CCl_4 may be found at high levels in raw and drinking water. After a chemical manufacturer accidentally spilled an estimated 70 tons of CCl_4 into the Kanawha River, the U.S. EPA determined that raw Ohio River water contained CCl_4 levels up to 0.340 mg/ℓ; drinking water levels were found to be as high as 0.1 mg/ℓ (Landen, 1979). As indicated by this incident, levels of CCl_4 detected in ambient water should not be construed to be the same levels of CCl_4 in public drinking water supplies. Municipal water treatment methods remove some of the contaminant.

Carbon tetrachloride has been found in the ppb range or lower in samples of rain, surface water, potable water and seawater (McConnell et al., 1975). Trace levels of CCl_4 have been reported in snow (Su and Goldberg, 1976). Carbon tetrachloride was also detected in the Atlantic Ocean at mean levels of 60 ppt (±17 ppt). Ocean levels of CCl_4 were only slightly higher in the Northern Hemisphere than in the Southern Hemisphere (Lovelock et al., 1973).

Lake Zurich and the ground water in an industrial section of Zurich, Switzerland, were also monitored for CCl_4. At various depths of Lake Zurich, concentrations of this compound of ~25 ppt were measured, with no significant variation. Groundwater levels of CCl_4 in the industrial sector were much larger. The compound was detected in 4 of 18 samples at levels ranging between 190 and 3600 ppt (Giger et al., 1978).

Carbon tetrachloride can be emitted to the environment through the production and use of the chemical, and through the production and use of chlorofluorocarbons and other chlorinated compounds that contain CCl_4 impurities. Although small amounts of CCl_4 may be directly released to water systems through these processes, most of the CCl_4 emitted to the environment has been estimated to be released to air or land (Rams et al., 1979). Direct releases to water may not account for the CCl_4 eventually detected in water. The chemical may find its way from air or land to surface and groundwater systems through rainfall, runoff from agricultural sites, dumping sites or industrial sites, and landfill leaching.

High levels of CCl_4 in the ground waters of Zurich, Switzerland have been attributed to industrial processes in the Zurich area (Giger et al., 1978). Levels of CCl_4 would be expected to be highest in industrialized areas because of both industrial and consumer use of CCl_4 and its products in these areas.

Carbon tetrachloride does not appear to be produced in water through chlorination reactions, unlike other chlorinated organics such as chloroform (Federal Register, 1979). Recent high levels of CCl_4 detected in Philadelphia drinking water following chlorination ($\leq$0.046 mg/ℓ) were found to be the result of the use of CCl_4-contaminated chlorine. This incident resulted in a meeting between U.S. EPA and the Chlorine Institute during which an interim maximum level for CCl_4 in chlorine for drinking water use was set at 0.1 mg/ℓ. The use of chlorine of this purity should result in CCl_4 levels of <0.001 mg/ℓ in drinking water (U.S. EPA, 1977).

4.3.2. Possible Sources and Levels of Carbon Tetrachloride in Air.

Reported concentrations of CCl_4 in continental and marine air masses in 1978 were very similar (0.00070-0.00084 mg/m³) (Table 4-1). As reported by Lovelock et al. (1973), the levels of CCl_4 in the Southern Hemisphere were only slightly lower than those in the Northern Hemisphere. The homogeneity of ambient CCl_4 concentrations throughout the atmosphere has been attributed to its slow and continual release to the atmosphere for many years (Federal Register, 1980a).

In general, CCl_4 levels over urban areas are only slightly higher than background levels (see Table 4-1). However, some higher concentrations have been reported. An average annual concentration of 0.0088 mg/m³ was reported over Tokyo in 1974-1975 (Ohta et al., 1976). This level was the highest measured over an extended period of time. Other high ambient concentrations measured reflected one sample or several covering a short time period (NAS, 1978). The maximum concentration ever detected was 0.113 mg/m³ in Bayonne, New Jersey (Lillian et al., 1975). A CCl_4 concentration of >0.0094 mg/m³ was also detected in the air of Grenoble, France (Su and Goldberg, 1976). Singh et al. (1980) measured CCl_4 concentrations in seven U.S. cities over 2-week periods (Table 4-2). The highest level detected using GC/ECD was 0.0188 mg/m³ over Houston, Texas.

TABLE 4-1

Summary of Atmospheric Concentrations of Carbon Tetrachloride*

Type of Measurement	Carbon Tetrachloride mg/m³
Continental background	0.00076 ± 0.00008
	0.00084 ± 0.00006
	0.00073 ± 0.00005
	0.00075 ± 0.00009
Marine background	0.00081 ± 0.00003
	0.00081 ± 0.00010
	0.00070 ± 0.00007
Urban range	0.00084 ± 0.00012
	0.00075 ± 0.113
	0.0088
	0.00075 ± 0.0094

*Source: NAS, 1978

TABLE 4-2

Atmospheric Concentrations of Carbon Tetrachloride Over Seven U.S. Cities*

City/State	Sampling Dates	CCl_4 (mg/m^3)		
		Mean	Maximum	Minimum
Los Angeles, CA	April 9 - April 21, 1979	0.0014	0.0064	0.0006
Phoenix, AZ	Apr. 23 - May 6, 1979	0.0018	0.0055	0.0008
Oakland, CA	June 28 - July 10, 1979	0.0011	0.0063	0.0006
Houston, TX	May 14 - May 25, 1980	0.0026	0.0188	0.0008
St. Louis, MO	May 29 - June 6, 1980	0.0008	0.0009	0.0007
Denver, CO	June 15 - June 28, 1980	0.0011	0.0018	0.0007
Riverside, CA	July 1 - July 13, 1980	0.0011	0.0017	0.0010

*Source: Singh et al., 1980

Carbon tetrachloride in the atmosphere appears to be of anthropogenic origin, because estimates of releases of CCl_4 from industrial processes and uses appear to account for the amount present in the atmosphere (Singh et al., 1976; Penkett et al., 1979). In 1978, ~2 million kg (~4.5 million pounds) of CCl_4 were emitted from production facilities. The total nationwide emissions of CCl_4 in 1978 from all sources were estimated at 29 million kg (65 million pounds). The primary source of these emissions is solvent application.

4.3.3. Possible Sources and Levels of Carbon Tetrachloride in Food.

In British studies, CCl_4 was found in various foods as follows (McConnell et al., 1975; Pearson and McConnell, 1975):

	Concentration (mg/kg)
Dairy products	0.0002-0.014
Meat	0.007-0.009
Oils and fats	0.0007-0.018
Beverages	0.0002-0.006
Fruits and vegetables	0.003-0.008
Black grapes (imported)	0.0197
Fresh bread	0.005
Fish and seafood	0.0001-0.006

The best studied mechanism of CCl_4 contamination in food is its use as a fumigant for grain. Much higher CCl_4 concentrations than those previously discussed have been found in fumigated grains. Residues as high as 115 mg/kg in wheat and 21 mg/kg in flour were detected by Ramsey (1957) through work on methods development following fumigation with a normal dosage of CCl_4-CS_2. The author states that the results "are not highly accurate" since they were obtained during work on development of methods.

Higher values (up to 150 mg/kg in wheat following 3 hours of aspiration) were detected by Mapes and Shrader (1957). However, it is not clear if the doses administered were normal for commercial fumigation. Carbon tetrachloride levels decline dramatically (dependent on time of aeration) in bread baked from fumigated wheat with residual levels generally reported as ≤10 mg/kg (U.S. EPA, 1980a; Jagielski et al., 1978). However, Berck (1974) reported that bread made from wheat aerated for 3 days had residues ~0.04 mg/kg in the upper and lower crusts and 0.13 mg/kg in the crumbs. In bread made from wheat aerated for 7 weeks, the upper crust had no residue, the lower crust had 0.2 mg/kg, and the crumbs had 0.01 mg/kg. The studies of CCl_4 levels in fumigated grains and grain products are discussed below.

Wheat, corn and milo fumigated and stored for 1-6 months were shown to contain CCl_4 at 2.9-20.4 mg/kg. The levels detected appeared to be dependent upon length of storage and concentration of fumigants (McMahon, 1971). In a monitoring program for grain imported into the Netherlands, CCl_4 was detected in half of the cereals sampled. Of these samples, 3% contained >5 mg/kg CCl_4 (Wit, 1972). The U.S. EPA Pesticide Laboratory detected 0.005-2.61 mg/kg CCl_4 in flour from 11 U.S. cities, with an average level of 0.051 mg/kg (Federal Register, 1980a). Carbon tetrachloride levels of 0.0002-0.0003 mg/kg were detected in flour in another study. However, bread and biscuits made from this flour contained undetectable CCl_4 of <5 mg/kg (Bondi and Alumot, 1972).

In several experiments, researchers have simulated commercial fumigation conditions to determine residual levels of CCl_4 in foods. Fumigated wheat, aerated for several weeks, was found to contain 20-62 mg/kg CCl_4.

Flour made from this wheat was found to contain 2-10 mg/kg CCl_4, whereas white bread made from the flour contained $\leq$0.007 mg/kg (Wit, 1972). In another study, CCl_4 at 200-400 mg/kg was detected in wheat and corn after application of a fumigant (Scudamore and Heuser, 1973). Residual CCl_4 decreased to 1-10 mg/kg 6 months after fumigation. By 12 months after fumigation, the wheat and corn contained a maximum of 4.7 mg/kg CCl_4. Wheat and barley were analyzed for CCl_4 in a study by Bielorai and Alumot (1966). The initial CCl_4 concentrations of 1.53 mg/kg in wheat and 2.2 mg/kg in barley decreased to 0.7 and 0.6 mg/kg, respectively, by day 17.

Carbon tetrachloride was detected in levels of 76-115 mg/kg in wheat, 10-21 mg/kg in flour, 28-39 mg/kg in oats and 43-88 mg/kg in bran that had been fumigated with recommended fumigant dosages (Lynn and Vorches, 1957). In another study, CCl_4 levels were determined in fumigated wheat and wheat fractions and in bread prepared from the wheat (Berck, 1974). Levels in wheat ranged between 3.2 mg/kg (7 weeks of aeration) and 72.6 mg/kg (1 week of aeration). Flour, bran and middlings contained 0.2-0.93, 0.43-3.53 and 0.2-1.65 mg/kg, respectively. Bread contained $\leq$0.13 mg/kg CCl_4. In a similar study, flour treated at normal fumigant levels was found to contain CCl_4 at levels of 0.6-1.6 mg/kg; levels in bran were 2.9-5.3 mg/kg (Jagielski et al., 1978). Bread baked from the flour contained <0.01 mg/kg CCl_4. Results of these studies indicated that the amount of CCl_4 remaining as a residue is dependent upon the fumigant dosage, storage conditions, length of aeration and extent of processing.

In addition to its use as a grain fumigant, CCl_4 may find its way into other foods through the use of herbicides, insecticides and fungicides containing CCl_4 as a contaminant (0.18-0.4%) in the pesticide formulation. Food may also become contaminated by CCl_4 in the air (Federal Register, 1980a).

4.3.4. Possible Sources and Levels of Carbon Tetrachloride in Soil. Carbon tetrachloride can occur in soil due to spills, runoff and leaching. As in groundwater contamination, CCl_4 may find its way into the soil by runoff from agricultural, dumping and industrial sites and through landfill leaching.

Wastewater treatment of night soil in Japan also resulted in CCl_4 formation. Methanol (MeOH)-water-solution substance was fractionated along with humic, fulvic and hymatomelanic acids from the effluent of the night soil treatment plant by the use of Amberlite XAD-2. Each of these four fractions was chlorinated at pH 7.0. Although all fractions primarily produced $CHCl_3$, CCl_4 was formed by chlorination of the MeOH-water-solution substance (Ishikawa et al., 1978).

4.4. RELATIVE SOURCE CONTRIBUTIONS

The widespread distribution of CCl_4 in the environment can lead to exposure to the chemical through water, food and air. Quantities of CCl_4 potentially taken into the body as a result of exposure to CCl_4 in air, water and food were estimated by the National Academy of Sciences (1978). Occupational exposure to CCl_4 was not considered in these estimates. The NAS computations were based upon data for fluid consumption and respiratory volume for reference individuals and worldwide per capita food consumption as compiled by the International Commission for Radiological Protection (ICRP, 1975). These data are shown in Tables 4-3 through 4-7.

Data on measured fluid intakes for adults and individuals are presented in Table 4-3, and calculated intakes of milk, water and other fluids for a typical (reference) man, woman, 10-year-old child and 1-year-old infant are given in Table 4-4. Respiratory volumes for these reference individuals are reported in Table 4-5. Food consumption per capita is reported by geographical regions in Table 4-6; the worldwide profile is presented in Table 4-7.

TABLE 4-3

Measured Fluid Intakes[a]

	Total Fluids[b]		Milk		Tap Water		Water-Based Drinks[c]	
	(mℓ/day)	(ℓ/yr)	(mℓ/day)	(ℓ/yr)	(mℓ/day)	(ℓ/yr)	(mℓ/day)	(ℓ/yr)
Adults (normal conditions)	1000-2400	365-876	120-450	44-164	45-730	16.4-266	320-1450	117-529
Adults (high environmental temperature to 32°C)	2840-3410 3256±900	1037-1245 1188±329	-	-	-	-	-	-
Adults (moderately active)	3700	1351	-	-	-	-	-	-
Children (5-14 yrs)	1000-1670	365-610	330-650	120-237	540-790 mℓ/day[d] 197-288 ℓ/yr[d]			

[a] Source: Adapted from ICRP, 1975

[b] Numbers in these columns are independent measurements, not totals from the following six columns. Totals of milk, tap water, and water-based drinks, therefore, do not correspond to total fluid values.

[c] Includes tea, coffee, soft drinks, beer, cider, wine, etc.

[d] Combined tap water and water-based drinks.

TABLE 4-4

Fluid Intake for Reference Individuals*

	Adult Man		Adult Woman		Child, 10 yr	
	(mℓ/day)	(ℓ/yr)	(mℓ/day)	(ℓ/yr)	(mℓ/day)	(ℓ/yr)
Milk	300	109.5	200	73.0	450	164.3
Tap water	150	54.8	100	36.5	200	73.0
Other	1500	547.5	1100	401.5	750	273.8
Total fluid	1950	711.8	1400	511.0	1400	511.0

*Source: Adapted from ICRP, 1975

TABLE 4-5

Respiratory Volumes for Reference Individual*
(in liters of air breathed)

	Adult Man	Adult Woman	Child, 10 years	Infant, 1 year
8 hours working, light activity	9600	9100	6240	2500 (10-hr)
8 hours nonoccupational	9600	9100	6240	
8 hours resting	3600	2900	2300	1300 (14-hr)
Total per day	2.3×10^4	2.1×10^4	1.5×10^4	0.38×10^4
Total per year	8.4×10^6	7.7×10^6	5.5×10^6	1.4×10^6

*Source: Adapted from ICRP, 1975

TABLE 4-6

Per Capita Estimates of World Food Consumption by Region[a] (g/day)

Food Group	Far East	Near East	Africa	Latin America	Europe	North America	Oceania
Cereals[b]	404	446	330	281	375	185	243
Starchy roots[c]	156	44	473	247	377	136	144
Sugar[d]	22	37	29	85	79	113	135
Pulses and nuts[e]	56	47	37	46	15	19	11
Vegetables and fruits[f]	128	398	215	313	316	516	386
Meat[g]	24	35	40	102	111	248	312
Eggs[h]	3	5	4	11	23	55	31
Fish[i]	27	12	16	18	38	26	22
Milk[j]	51	214	96	240	494	850	574
Fats and oils[k]	9	20	19	24	44	56	45

[a] Source: Adapted from ICRP, 1975
[b] Flour and milled rice content
[c] Includes sweet potatoes, cassava and other edible roots
[d] Includes raw sugar; excludes syrups and honey
[e] Includes cocoa beans
[f] Fresh equivalent
[g] Includes offal, poultry and game expressed as carcass weight, excluding slaughter fats
[h] Fresh egg equivalent
[i] Landed weight
[j] Excludes butter; includes milk products as fresh milk equivalent
[k] Pure fat content

TABLE 4-7

Summary of Per Capita Estimates of World Food Consumption[a]

Food Groups	Worldwide Minimum (g/day)	Maximum (g/day)	Minimum (kg/yr)	Maximum (kg/yr)
Cereals[b]	185	446	67.5	162.8
Starchy roots[c]	44	473	16.1	172.6
Sugar[d]	22	135	8.0	49.3
Pulses and nuts[e]	11	56	4.0	20.4
Vegetables and fruits[f]	128	516	46.7	188.3
Meats[g]	24	312	8.8	113.9
Eggs[h]	3	55	1.1	20.1
Fish[i]	12	38	4.4	13.9
Milk[j]	51	850	18.6	310.2
Fats and oils[k]	9	56	3.3	20.4

[a] Source: Adapted from ICRP, 1975

[b] Flour and milled

[c] Includes sweet potatoes, cassava and other edible roots

[d] Includes raw sugar; excludes syrups and honey

[e] Includes cocoa beans

[f] Fresh equivalent

[g] Includes offal, poultry and game expressed as carcass weight, excluding slaughter fats

[h] Fresh egg equivalent

[i] Landed weight

[j] Excludes butter; includes milk products as fresh milk equivalent

[k] Pure fat content

The data in these tables summarize human fluid, respiratory and food intake. NAS combined these intake data with information on CCl_4 contamination of water, air and food to estimate human exposure to CCl_4. A summary of the NAS computations of CCl_4 intake follows.

4.4.1. **Water.** The potential CCl_4 uptake from fluids is shown in Table 4-8. NAS used in its calculations the levels of CCl_4 reported in drinking water in the NORS study (Symons et al., 1975). These levels were <0.003 mg/ℓ. In a later report (the NOMS) CCl_4 was detected at levels of 0.0024-0.0064 mg/ℓ in U.S. drinking water (U.S. EPA, 1980a). Estimation of CCl_4 consumption derived by using the NOMS maximum value of 0.0064 mg/ℓ were included in the data in Table 4-8. These data were calculated by multiplying the minimum and maximum concentrations in drinking water by the minimum, maximum and "reference" consumption of drinking water. These calculations were based upon the following assumptions:

1. 100% of the CCl_4 ingested is absorbed.
2. Commercially-produced drinks and drinks reconstituted in the home contain the same level of CCl_4 as does drinking water.
3. All fluids contain the indicated CCl_4 concentration.

Values for total fluid uptake include drinks such as milk, not prepared by mixing with water.

4.4.2. Air. The potential CCl_4 uptake from air is presented in Table 4-9. The minimum level for CCl_4 in air used by NAS was 0.00075 mg/m³, the typical level was 0.00094 mg/m³ and the maximum level was 0.113 mg/m³, reported by Lillian et al. (1975) for a monitoring sample for Bayonne, New Jersey. These values were multiplied by the average quantity of air inhaled per year (from Table 4-5) to arrive at the average quantity of CCl_4 inhaled per year. In the NAS report, this quantity was then

TABLE 4-8

Carbon Tetrachloride Uptake from Fluids (mg/yr) Calculated by Assuming 100% Absorption[a]

Exposure	Fluid	Adult Man, Fluid Intake			Adult Woman, Fluid Intake			Child, Fluid Intake		
								5-14 years		10 years
		Minimum	Maximum	Reference	Minimum	Maximum	Reference	Minimum	Maximum	Reference
Minimum	Tap water	0.03	0.53	0.11	0.03	0.53	0.07		0.58	0.15
(0.002 mg/ℓ)[c]	Other[b]	0.23	1.06	1.10	0.23	1.06	0.80			0.55
	Total fluid	0.73	2.70	1.42	0.73	2.70	1.02	0.73	1.22	1.02
Maximum	Tap water	0.05	0.80	0.16	0.05	0.80	0.11	0.59	0.86	0.22
(0.003 mg/ℓ)[c]	Other[b]	0.35	1.59	1.64	0.35	1.59	1.20			0.82
	Total fluid	1.10	4.05	2.14	1.10	4.05	1.53	1.10	1.83	1.53
Maximum	Tap water	0.10	1.70	0.35	0.10	1.70	0.23	1.26	1.84	0.47
(0.0064 mg/ℓ)[d]	Other[b]	0.75	3.39	3.50	0.75	3.39	2.57			1.75
	Total fluid	2.34	8.65	4.56	2.34	8.65	3.27	2.34	3.90	3.27

[a] Adapted from NAS, 1978

[b] Includes water-based drinks such as tea, coffee, soft drinks, beer, cider, wine.

[c] Calculated by multiplying the exposure concentration (mg/ℓ) x fluid intake (ℓ/yr) (from Table 4-3 for minimum and maximum intakes and Table 4-4 for reference intakes).

[d] Similar calculations based upon NOMS maximum drinking water levels of 6.4 μg/ℓ.

TABLE 4-9

Estimated Human Uptake of Carbon Tetrachloride from the Outdoor Atmosphere[a,b]
(mg/yr)

	Adult Man, Exposure			Adult Woman, Exposure		
	Minimum	Typical	Maximum	Minimum	Typical	Maximum
Average inhaled[c]	6.3	7.9	951	5.8	7.3	872
Minimum absorbed[d]	3.6	4.5	542	3.3	4.1	457
Maximum absorbed[d]	4.1	5.2	618	3.8	4.7	567
	Child, 10 yr, Exposure			Infant, 1 yr, Exposure		
Average inhaled[c]	4.2	5.2	623	1.1	1.3	159
Minimum absorbed[d]	2.4	3.0	355	0.6	0.6	90.0
Maximum absorbed[d]	2.7	3.4	405	0.7	0.9	103

[a] Source: Adapted from NAS, 1978

[b] The absorption range used is that reported by Lehmann and Schmidt-Kehl (1936). Based upon human data, the range given is 57-65%, calculated from the concentration of CCl_4 in the inhaled air and that found in the exhaled breath.

[c] Calculated by applying respiratory volume per year (see Table 4-5) to the general range of atmospheric concentrations.

[d] Calculated by applying the range of percent absorptions to the total amount inhaled per year.

multiplied by the minimum and maximum values of the absorption range for CCl_4 in air as reported for humans by Lehmann and Schmidt-Kehl (1936). These calculations resulted in values for the minimum and maximum yearly absorption of CCl_4 from air. The issue of percent absorption of CCl_4 has not been clearly defined. As discussed in Section 7.1., the percent absorbed may vary between species. The range reported by Lehmann and Schmidt-Kehl and used in the NAS report, is retained here since it is the only human data available. See Section 7.1. for a more detailed discussion on absorption of CCl_4.

4.4.3. Food. The potential CCl_4 uptake from foods is presented in Table 4-10. Values of CCl_4 found in foods and used in these calculations are presented in Table 4-11. To arrive at uptake values, the minimum and maximum levels of CCl_4 in foods were multiplied by the minimum and maximum worldwide food intake for individuals for each food category. NAS calculated a per capita uptake of 0.21-7.33 mg CCl_4 from food each year.

The NAS absorption calculations did not include the yearly uptake through the consumption of bread. The average consumption of CCl_4 attributed to bread has been calculated elsewhere as 777 ng/day or 0.3 mg/yr (Federal Register, 1980a). The data on consumption in bread are included in the summary of CCl_4 uptake from all sources (Table 4-12).

4.4.4. Soil. The potential uptake of CCl_4 from soil is unknown. This includes agricultural runoff as well as uptake from plants.

4.5. SUMMARY

Carbon tetrachloride is produced commercially from the chlorination of methane, propane, ethane propylene and carbon disulfide. Production has declined over the last 10 years and a 1.0%/year decline is projected through 1985.

TABLE 4-10

Carbon Tetrachloride Uptake from Food Supplies (mg/yr), Calculated by Assuming 100% Absorption[a,b]

Food Group[c]	Worldwide Food Intake[d]	Exposure		
		Minimum	Average	Maximum
Milk products	Minimum	0.004	--	0.26
	Maximum	0.06	--	4.34
Eggs	Minimum	--	0.001	--
	Maximum	--	0.01	--
Meats	Minimum	0.06	--	0.08
	Maximum	0.80	--	1.02
Fats and oils	Minimum	0.002	--	0.06
	Maximum	0.01	--	0.37
Vegetables and fruits	Minimum	0.14	--	0.37
	Maximum	0.56	--	1.51
Fish and seafood	Minimum	<0.001	--	0.03
	Maximum	0.001	--	0.08
Total, all food supplies	Minimum	0.21	--	--
	Average	--	1.12	--
	Maximum	--	--	7.33

[a] Source: Adapted from NAS, 1978

[b] Calculated by applying worldwide food intake ranges for various food groups (see Table 4-7) to the range of concentrations found in various foods (see Table 4-10).

[c] As used in Tables 4-6 and 4-7.

[d] From Table 4-7.

TABLE 4-11

Summary of Carbon Tetrachloride Concentrations in Food Supplies* (ppb by weight, $\mu g/kg$)

Food Group	Carbon Tetrachloride		
	Minimum	Average	Maximum
Milk products	0.2	--	14.0
Eggs	--	0.5	--
Meats	7.0	--	9.0
Fats and oils	0.7	--	18.0
Vegetables and fruits	3.0	--	8.0
Fish and seafood	0.1	--	6.0

*Source: Adapted from McConnell et al., 1975; Pearson and McConnell, 1975

TABLE 4-12

Relative Uptake of Carbon Tetrachloride from the Environment by Adult Male[a]

Source	Minimum Exposure[b]		Typical Exposure[c]		Maximum Exposure[d]	
	(mg/yr)	(% of total)	(mg/yr)	(% of total)	(mg/yr)	(% of total)
Fluid intake	0.73	16	3.13	34	8.65	1
Atmosphere	3.60	79	4.75	51	618	98
Food supply	0.21	5	1.42	15	7.63	1
Total	4.54	--	9.30	--	634.28	

[a] Source: Adapted from NAS, 1978

[b] For minimum conditions: assume minimum intake for fluids, minimum absorption from atmosphere, and minimum intake for food supplies.

[c] For typical conditions: assume reference male intake for fluids, 0.0044 mg/ℓ as average of concentrations in NOMS study; for intake from atmosphere, assume average of minimum and maximum absorption rates for typical exposure; for intake from food supplies, assume average exposure and intake from NAS, 1978 plus 0.3 mg/yr from bread consumption (Federal Register, 1980a).

[d] For maximum conditions: assume maximum intake for fluids, CCl_4 at 0.0064 mg/ℓ as in NORS study; maximum absorption from atmosphere; and maximum intake from food from NAS, 1978 plus 0.3 mg/yr from bread consumption (Federal Register, 1980a).

Carbon tetrachloride is ubiquitous in the environment and has been detected in concentrations of generally <0.01 mg/ℓ in water, <0.01 mg/m³ in air and <0.01 mg/kg in food. Higher levels of CCl_4 have been detected in urban air, and in grain and food products made from grain. Increased levels of CCl_4 in urban air are probably due to the industrial use of CCl_4 and products containing CCl_4. The presence of CCl_4 in grains or grain products is due in part to the use of fumigants containing CCl_4.

Data on the uptake of CCl_4 by the adult male are summarized in Table 4-12. At minimum exposure levels, uptake from the air appears to be the major source of exposure (79%), followed by fluids (16%) and the food supply (5%). At typical exposure levels, uptakes from fluid (34%) and food (15%) appear to increase with respect to that from air (51%). At maximum exposure levels, 98% of CCl_4 uptake is estimated to be from air. Amount of uptake from soil is unknown.

All of the CCl_4 in the environment can be accounted for by anthropogenic activities. Carbon tetrachloride, unlike chloroform and other halocarbons, does not appear to be indirectly formed during water chlorination. No natural sources of CCl_4 are known.

5. FATE AND TRANSPORT

5.1. FATE

5.1.1. Water. Carbon tetrachloride tends to evaporate from dilute aqueous solutions, with a half-life of only 29 minutes (Dilling et al., 1975). Chemical stability in aquatic environments is high, with half-lives of $7 \times 10^{3}/C$ years, where C is concentration in mg/ℓ. This formula is derived from the second order rate constant of $4.8 \times 10^{-7}/mol^{-1}/sec^{-1}$ (Mabey and Mill, 1978). Singh et al. (1976) give an approximate hydrolytic half-life for CCl_4 in oceans as 7×10^{4} years. This figure is lower than that predicted using measured ocean concentrations (Pearson and McConnell, 1975) and the above second order formula. For the maximum Atlantic Ocean concentration of 2.4×10^{-6} mg/ℓ, the half-life is 2.9×10^{6} years. In any case, CCl_4 is extremely stable in water, with losses primarily due to other factors such as evaporation, sediment adsorption and organism uptake. Singh et al. (1976) estimate that 2-3% of all biospheric CCl_4 is in solution. Although CCl_4 is not easily transported to groundwater due to its high volatility, low solubility and low mobility in soil, any contamination is likely to persist for many years and accumulate.

5.1.2. Air. Chemical stability, a long lifetime, and uniform mixing characterize the fate of CCl_4 in air. Mixing is assumed to be uniform to approximately 18 km elevation, with a pseudo-first order removal rate of 1×10^{-3} yr^{-1}, mainly via gas-phase reactions involving the electronic state $O(^{1}D)$ (Galbally, 1976). The principal sink is considered to be the stratosphere (Galbally, 1976; Singh et al., 1976), with significant loss of CCl_4 limited by the rate of transport from the troposphere. Above 18 km, stratospheric photolysis is dominant. This photodissociation is attributed to UV radiation mainly in the 195-225 nm region.

Simulated tropospheric irradiation produces no discernible dissociation of CCl_4 in NO_2-air mixtures. In a laboratory experiment designed to detect stratospheric reactivity of stable halocarbons, Lilian et al. (1957) determined the minimum stratospheric half-life of CCl_4 to be 37 hours. This corresponds to a lifetime (time to reach 1/e of initial concentration) of 53 hours. Golombeck (1982) estimated a stratospheric lifetime of 7.5 years using a global three-dimensional model. Using the same model, the atmospheric lifetime was estimated at ~50 years. Estimates of atmospheric lifetime are also quite variable ranging from 18 years (Krey et al., 1976) to "several decades" (Galbally, 1976) and 60-100 years (Singh et al., 1976).

Carbon tetrachloride in the stratosphere is considered to be a potential source of chlorine via photodissociation which may, on a molecule-by-molecule basis, have the potential to catalyze the destruction of the ozone layer approximately as strong as CFC-11 and CFC-12 (Hanst, 1978; NAS, 1978). Following a relatively rapid tropospheric mixing with other halogenated methanes, there is a relatively slow entry of CCl_4 into the stratosphere, followed by a random ascent to altitudes (>25 km) where solar ultraviolet (UV) radiation in the range 185-225 nm photodissociates CCl_4 resulting in the generation of "odd chlorine" (i.e., a variety of chlorine-containing species) (NAS, 1978). Dependent upon the rates of a variety of chemical reactions and the rate of odd chlorine to the troposphere, each odd chlorine is responsible for the destruction of several thousand ozone molecules. One projection is that CCl_4, along with hydrochloric acid (HCl) and methyl chloride (CH_3Cl), together contribute to a reduction of less than 1% of stratospheric ozone, comparable to but less than the rate of ozone reduction attributed to chlorofluoromethanes. The relative importance of HCl and CH_3Cl is reduced, however, due to atmospheric "rainout" and degradative processes, respectively (NAS, 1978). Based on the data, assumed release rates and depletion models discussed in NAS (1984) and Hudson et al. (1982) the steady-state decrease in the ozone layer due to CCl_4 alone can be estimated at 0.3-0.5%.

The future impact of CCl_4 emissions on stratospheric ozone is presently difficult to predict, as is the collective impact of all chlorocarbons (CLC) on stratospheric ozone. Significant advances in the chemistry and modeling aspects (Sugdon and West, 1980) during the past decade concerning pertinent atmospheric processes, reviewed by NAS (1984), have led to much improved modeling estimates of potential decrements in total column atmospheric ozone illustrated in Figure 5-1.

Uncertainties in several areas remain to be resolved. Varying assumptions (Table 5-1) can be made regarding important parameters that determine rates of atmospheric conversions of CCl_4 and other chlorocarbons, and regarding their expected furture global emission rates. Anticipated changes in atmospheric levels of other compounds (e.g., methane, CO, NO_x, OH) could result in increases or decreases in the ozone layer. Thus several different scenarios are possible for future impacts of CCl_4 and other chlorocarbons on stratospheric ozone (see Section 6.3.1. and NAS, 1984). In particular, the roles of other chemical species such as CO_2, N_2O and H_2O and their combined effects with chlorinated hydrocarbons are not well understood (NAS, 1982; Wuebbles and Chang, 1981). It is believed that the combined effects are not additive, so specific cases would need to be investigated in order to make a precise prediction of future ozone reduction. Second, the various chemical cycles involved seem to be quite dependent on altitude. Thus, although several of the specific rate constants for the

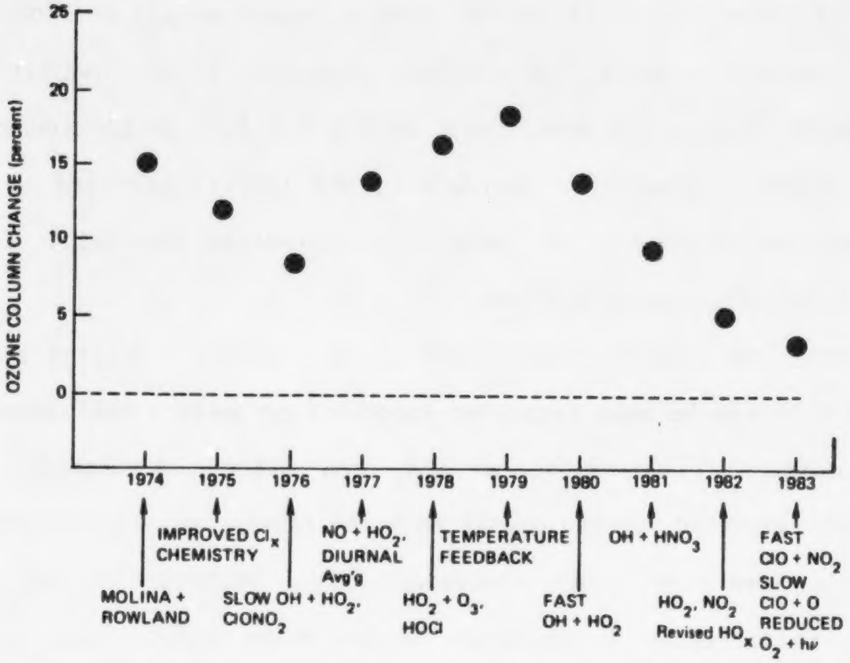


FIGURE 5-1

Estimates of steady-state reductions in total column ozone for continuous releases of CFCs at approximately 1975 rates as calculated from different chemical models. Changes in the models and simulation techniques are indicated in chronological order.

Source: NAS, 1984

TABLE 5-1

Uncertainties in Quantifying the Ozone Depletion Caused by CCl_4

Theory	Data
Transport models including model formulation and numerical accuracy	Measurement of photochemical reactions
Chemical mechanisms and kinetics for ozone destruction and influences of CCl_4 and non-CFC compounds	Normal year-to-year fluctuations in meteorological data (wind and temperature) and in stratospheric ozone distribution
Profile of ozone depletion vs. altitude, especially >40 km.	
Spatial and temporal dependence of water vapor exchange between troposphere and stratosphere	Emission data and projections for CCl_4, CFCs and other non-CFCs.

chlorine and nitrogen reactions have much improved estimates, the net result regarding eventual ozone reduction though understood, has several uncertainties.

5.1.3. Soil. Pertinent information concerning the degradation or transformation of CCl_4 in soil could not be located in the available literature. Galbally (1976) calculates that CCl_4 in all land biota is probably <0.2% of that in the atmosphere and concludes that land biota are an insignificant sink for CCl_4.

5.2. TRANSPORT

5.2.1. **Water.** Carbon tetrachloride introduced into water resources will be transported by movement of surface and ground water, and by evaporation into the atmosphere. Studies in England show transfer of CCl_4 from the atmosphere to surface water due to heavy rainfall (McConnell, 1976). In dilute aqueous solutions, the evaporative half-life is 29 minutes (Dilling et al., 1975). However, large, concentrated quantities tend to remain as one mass or "slug". A spill into the Ohio River of ~70 tons of CCl_4 produced a slug estimated to be 74 miles long (U.S. Congress, 1977). Degradation of CCl_4 by hydrolytic reactions is slow; when considered to be approximately first-order, the estimated half-life is 70,000 years (Johns, 1976). Thus, volatilization is considered to be the main process for removing CCl_4 from aquatic systems.

5.2.2. **Air.** The high vapor pressure, low water solubility and high specific gravity of CCl_4 influence its disposition in air environments. Volatilization to the atmosphere from land and water should be rapid. Actual transport in the air, however, has been infrequently demonstrated. Penkett et al. (1979) found higher atmospheric values over Great Britain during easterly winds than during westerly winds, reflecting the difference in anthropogenic activity under the two trajectories. Other reports show minor global variation in concentration of CCl_4 (Singh et al., 1976; Lovelock et al., 1973). Vertical transport from the lower atmosphere to the stratosphere accounts for ~25% of the atmospheric losses of CCl_4, assuming an overall atmospheric lifetime of 75 years and a quasi-steady state loading (Singh et al., 1976). Neely (1977) modeled the transport of CCl_4 vertically from the ocean through the troposphere to the stratosphere accounts for roughly 25% of the atmospheric losses of CCl_4, and horizontally between northern and southern hemispheres. Transfer between hemispheres was considered substantial, with a first-order rate constant in each direction of 0.9 yr^{-1}. Neely's simulation results agreed well (within 20%) with observed concentrations.

5.2.3. Soil. There is little quantitative information about adsorption of CCl_4 onto sediments. Using the approach of Briggs (1973), the sorption potential can be estimated by the following formula:

$$\log Q = 0.524 \log P + 0.618$$

where P is the octanol/water partition coefficient and Q is the organic matter/water partition coefficient. For CCl_4, log P = 2.64, and thus Q = 100.3. This value suggests that CCl_4 has "low" mobility in soil, as defined by Briggs (1973), indicating that it can move with soil, sediment or particulate matter. Morris and Johnson (1976) state that periods of high agricultural runoff (river turbidity) are directly correlated with high chloroform concentration in finished water and imply that similar associations hold for CCl_4 and haloforms in general. Statistical analysis of their data, however, demonstrates poor linear correlation (r = 0.06) between river turbidity and CCl_4 levels, with strong peaks occurring during periods of both low and high turbidity. Pearson and McConnell (1975) investigated chlorinated hydrocarbons in marine environments, but show no significant relationships between levels in sediment and levels in overlying seawater, sediment particle size or geographic features. More research is clearly needed on transport via rainfall, directly from the atmosphere and indirectly through agricultural runoff, in order to assess the importance of soil for CCl_4.

5.3. BIOACCUMULATION/BIOCONCENTRATION

The data of Kopperman et al. (1976) suggest that polar organochlorine compounds are easily biodegraded, while nonpolar, highly lipophilic compounds accumulate. Bioaccumulation is directly related to the octanol/water partition coefficient (P) of the compound (Neely et al., 1974). The log octanol/water partition coefficient (log P) of CCl_4 is 2.64 (Leo et al., 1971; Neely et al., 1974; Chiou et al., 1977), indicating a possible tendency for this compound to bioaccumulate under conditions of constant exposure. However, although CCl_4 and other organochlorines are lipophilic and tend to concentrate in fatty tissues, there is no evidence that they magnify through the food chain (Pearson and McConnell, 1975). Difficulties in the Pearson and McConnell (1975) study discourage estimates of bioaccumulation based on their results. Barrows et al. (1980) report a CCl_4 bioconcentration factor (BCF = tissue concentration divided by water concentration) of 30 for bluegill sunfish, Lepomis macrochirus, and a tissue half-life of <1 day. The authors state that the short tissue half-life makes it unlikely for CCl_4 to biomagnify in fish unless exposure is continuous or prolonged. Neely et al. (1974) estimate the steady state BCF for rainbow trout, Salmo gairdneri, to be 17. Data could not be found for bioconcentration of CCl_4 in shellfish.

5.4. SUMMARY

Transport and fate of CCl_4 have been studied most extensively in air, with some information on water levels and almost no data on soil. Carbon tetrachloride is extremely stable in water, the lower atmosphere and the troposphere. Photodissociation in the stratosphere is rapid in comparison to the atmospheric lifetime. Global distribution of CCl_4 in air is nearly uniform. Bioconcentration in fish is low. Research needs are most pronounced for fate in soil, transport to and fate in the stratosphere, and bioaccumulation/bioconcentration in shellfish.

6. ECOLOGICAL EFFECTS

Data on the ecological effects of CCl_4 are somewhat limited. The reasons for this can be inferred from the physical and chemical properties of the compound as discussed in Chapter 3. Carbon tetrachloride is quite volatile and so does not readily accumulate in either terrestrial or aquatic environments. It is also rapidly diluted to low concentrations in the troposphere. While major spills of CCl_4 have occurred, ecosystem effects have not been documented or considered to be significant enough as to merit further investigation (NAS, 1978). Apparent acute effects have been minimal and, consequently, chronic effects on fish and wildlife associated with long-term exposures at low levels are unlikely.

6.1. EFFECTS ON NONTARGET ORGANISMS

Because CCl_4 is used as a pesticide, primarily as a grain and soil fumigant, nontarget soil organisms are undoubtedly affected (NAS, 1978). The interaction of CCl_4 with anaerobic organisms was studied by Wood et al. (1968) using extracts of _Methanobacillus_ _omelianskii_ and the N^5-methyl tetrahydrofolate-homocysteine transmethylase of _Escherichia_ _coli_ _B_. Low concentrations of CCl_4 were found to inhibit cobamide-dependent methyl transfer reactions in these cell extracts. The possibility that CCl_4 might inhibit methyl transfer systems of microbial organisms in nature should be investigated, although there is no imminent hazard (NAS, 1978).

6.1.1. Aquatic Life Toxicology. The majority of the acute toxicity data for CCl_4 and aquatic organisms has been determined using static procedures with unmeasured test concentrations. Results of these tests may underestimate the acute toxicity of CCl_4 due to its volatility. No acute or chronic effects were observed at a concentration lower than 3400 μg/ℓ (U.S. EPA, 1978).

6.1.2. **Acute Toxicity.** As shown in Table 6-1, the 48-hour LC_{50}/EC_{50} for Daphnia magna is 35,200 μg/ℓ. In two separate studies, Dawson et al. (1977) determined a 96-hour LC_{50}/EC_{50} value for the bluegill of 125,000 μg/ℓ, and the U.S. EPA (1978) calculated the value to be 27,300 μg/ℓ. The reason for this large difference is not clear but may have been caused by the volatility of this compound. There appears to be no great difference in sensitivity between the two tested species. A flow-through test result for the fathead minnow is 43,100 μg/ℓ. However, no comment can be made concerning the effect of test conditions on the test results.

^{14}C-labeled CCl_4 (1 mℓ/kg, i.p.) produced a 5- to 10-fold increase in serum GOT, GPT, and ICD enzyme activities in rainbow trout, Salmo gairdneri, whereas exposure of trout to CCl_4 in tank water (1-80 mg/ℓ) produced neither mortality nor changes in serum enzyme activities. Tissue residue analysis revealed the highest concentration of CCl_4 is found in the adipose tissue followed by levels in the liver, brain, spleen and gills regardless of the administration route. Elimination rates of ^{14}C residues occurred at 2 hours for both i.p. and ambient water exposure routes totaling 4.8 and 0.75 μmol/g, respectively. Histologic examination of tissues revealed varying degrees of liver and splenic neurosis 6 hours following administration of CCl_4 (Statham et al., 1978).

Pfeifer and Weber (1981) treated rainbow trout with a single i.p. injection of CCl_4. Initially, fish were treated with 2.0 mℓ/kg CCl_4. At 3, 6, 12, 18, 24 and 36 hours post-treatment, blood samples were obtained and analyzed for total protein concentration. Additionally, the fish were treated with 0.25, 0.50, 1.0 or 2.0 mℓ/kg CCl_4 following which total

TABLE 6-1

Acute Values for Carbon Tetrachloride

Species	Method*	LC_{50}/EC_{50} (µg/ℓ)	Species Acute Value (µg/ℓ)	Reference
		FRESHWATER SPECIES		
Cladoceran *Daphnia magna*	S, U	35,200 (48-hour)	35,200	U.S. EPA, 1978
Fathead minnow *Pimephales promelas*	FT, M	43,100 (unknown)	43,100	Kimball, manuscript
Bluegill *Lepomis macrochirus*	S, U	125,000 (96-hour)	--	Dawson et al., 1977
Bluegill *Lepomis macrochirus*	S, U	27,300 (96-hour)	58,000	U.S. EPA, 1978
		SALTWATER SPECIES		
Tidewater silversides *Menidia beryllina*	S, U	150,000 (96-hour)	150,000	Dawson et al., 1977
Dab *Limanda limanda*	(estimated)	50,000 (96-hour)	--	Pearson and McConnell, 1975

*S = static, FT = flowthrough, U = unmeasured, M = measured

No Final Acute Values are calculable since the minimum data base requirements are not met.

plasma protein and albumin concentrations were measured at 24 hours post-treatment. The single injection of 2.0 mℓ/kg CCl_4 resulted in a significant decrease in total plasma protein concentration of rainbow trout at 12, 24 and 36 hours post-treatment. Plasma albumin was also reduced significantly at the 2.0 mℓ/kg dose level 24 hours post-treatment. The authors suggested that several factors, including intestinal inflammation and hemorrhage, may have contributed to the observed effects on plasma protein concentration.

In an earlier study using the same species, Pfeifer and Weber (1980) determined that a single i.p. dose of CCl_4 (2.0 mℓ/kg) produced either oliguria or anuria as early as 1 hour post-treatment. A significant reduction in urine flow as well as a significant increase in relative wet body weight were noted. Histological examination of kidney tissue at 24 hours post-treatment revealed early localized pathological changes, although extensive morphological damage was not evident. This, in conjunction with the early oliguria, suggests that the observed reduction in urine flow rate was not due to a direct toxic effect on the kidney.

Morphological changes in the liver of two species of carp, _Cyprino carpio_ and _Carassius_ _auratus_, were observed following i.p. injection of 0.3-5.0 mℓ/kg CCl_4 for at least 8 days (Jiang and Zhang, 1979). The following changes were noted in the liver tissue of both species: 1) proliferation in number of vacuoles, 2) nuclei enlargement in some cells, and 3) contraction and deformation of the nuclear membrane. These findings are in accord with those of studies discussed previously that reported decreased plasma protein concentrations and, consequently, reflect the hepatotoxicity of CCl_4.

Only two saltwater fish and no invertebrate species have been tested (Dawson et al., 1977). The 96-hour LC_{50}/EC_{50} for the tidewater silversides, Menidia beryllina, is 150,000 μg/ℓ (see Table 6-1). The other datum is an estimated 96-hour LC_{50} for the dab, Limanda limanda, of about 50,000 μg/ℓ.

6.1.3. Chronic Toxicity. No chronic test has been conducted with a freshwater invertebrate species or any saltwater species. An embryo-larval test with the fathead minnow, Pimephales promelas, resulted in no observable adverse effect at CCl_4 concentrations up to 3400 μg/ℓ (U.S. EPA, 1978).

6.2. TISSUE RESIDUES

The bluegill, Lepomis macrochirus, bioconcentrated CCl_4 at equilibrium to a factor of 30 times within 21 days (U.S. EPA, 1978). The biological half-life in these tissues was <1 day. In addition, Neely et al. (1974) exposed the rainbow trout to CCl_4 and estimated a steady-state bioconcentration factor (BCF) of 17. Similarly, Barrows et al. (1978) determined a BCF of 30 for bluegill sunfish. These results indicate that tissue residues of CCl_4 would not pose a potential environmental hazard to aquatic life.

6.3. INDIRECT ECOSYSTEM EFFECTS

Indirect ecosystem effects of CCl_4 (e.g., those caused by enhanced UV-B radiation) are similar to those of other halocarbons and result from photodissociation of the compound and subsequent destruction of stratospheric ozone (NAS, 1978, 1982). For more discussion of these effects, see Chapters 5 and 11.

6.3.1. Effect on Stratospheric Ozone. Carbon tetrachloride may contribute to an overall reduction in stratospheric ozone. Estimates of the actual amount of ozone depletion, even at steady-state, are surrounded by uncertainties. Several chemicals and conditions contribute to overall ozone reduction, and the combined influences are thought to be something other than strictly additive (NAS, 1982), making prediction of the effects difficult.

6.3.2. **Effect on UV Flux.** Changes in stratospheric ozone content result in alterations of UV flux to the earth's surface. This involves the biologically damaging wavelengths in the 290 to 320 nm range (UV-B regions). The molecular basis of the effects of these wavelengths is the alteration of protein and nucleic acid structures impacting both genetic replication and protein synthesis mechanisms (NAS, 1978). While there exist molecular repair processes that mitigate this damage, they may not be totally effective at all UV intensities.

Increased UV-B radiation adversely affects a variety of plant species in terms of depressed photosynthetic activity and reduced growth rate. Studies of both agriculturally significant plants and higher nonagricultural species (cited by NAS, 1978) demonstrate a diverse response to enriched levels of UV radiation. These findings include inhibition of seed germination and increased somatic mutation rates, in addition to depressed photosynthetic activity. Although effects on natural ecosystems are difficult to study and predict, the ecological impact of such effects may be significant to natural communities taken collectively, even if only a few constituent species are affected. In addition to the direct effect of UV-B wavelengths on these species, effects on interacting and/or dependent species must be considered (NAS, 1978) in assessing ecosystem effects.

6.4. SUMMARY

Only two freshwater fish and one invertebrate species have been acutely tested resulting in a 96-hour LC_{50} determined to be as low as 27,300 µg/ℓ. No definitive chronic data are available. Tissue residues of

CCl_4 do not appear to be a problem since available data suggest a BCF of <30.

The cited data for CCl_4 indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 35,200 μg/ℓ and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of CCl_4 to sensitive freshwater aquatic life.

The data also indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 50,000 μg/ℓ and would occur at lower concentrations among more sensitive species. As for freshwater aquatic life, no data are available concerning chronic toxicity to sensitive species.

Indirect effects of CCl_4 are associated with reduced levels of atmospheric ozone and concomitant increases of UV-B radiation flux. There are known differences in species sensitivities to increases in UV-B radiation. Laboratory studies have identified effects associated with such increases. At this time, the determination of the magnitude of effects of enhanced UV-B radiation collectively or for individual species in the natural environment is being studied.

7. COMPOUND DISPOSITION AND RELEVANT PHARMACOKINETICS

This chapter is divided into four sections: absorption, distribution, metabolism and excretion of CCl_4. The absorption section begins with a discussion of the chemical's partition coefficients. As would be expected for a compound with high partition coefficients for oil/air and oil/water, CCl_4 is reported to be absorbed readily through the lungs and also through the intestinal tract and skin. Once absorbed, the chemical and its metabolites are reported to be distributed widely throughout the body, with high concentrations in liver, bone marrow, blood, muscle, fat and brain. Several metabolites of CCl_4 have been identified, including chloroform, hexachloroethane and carbon dioxide. Carbonyl chloride has been postulated as a metabolite by inference based on an *in vitro* study of a $[^{14}C]CCl_4$ incubation system. Carbon tetrachloride metabolism has been proposed to involve a complex with ferrocytochrome heme and formation of the free radical CCl_3. Carbon tetrachloride and its metabolites are reported to be excreted principally in exhaled air but also in urine and feces.

7.1. ABSORPTION

7.1.1. Partition Coefficients. Partition coefficients for various chlorinated solvents, including CCl_4, were determined by several experiments (Morgan et al., 1972; Sato and Nakajima, 1979; Powell, 1945). The partition coefficient is a measure of the relative solubility of a substance in two media. The oil/air and oil/water partition coefficients can be used as indicators of solubility in lipids. The values of these and other partition coefficients for CCl_4, listed in Table 7-1, show this chemical to be lipophilic. Because of its lipophilic nature, one would predict that CCl_4 could be absorbed by ingestion, inhalation and skin contact. This prediction is borne out by results of the experimental studies described below.

TABLE 7-1

Partition Coefficients for Carbon Tetrachloride*

Parameter	Partition Coefficients		
	20°C	25°C	37°C
Olive oil/air	--	142	361
Blood serum/air	--	6	--
Blood/air	3.6-5.2	--	2.4
Water/air	--	0.25	--
Olive oil/water	--	1440	--
Olive oil/serum	--	23	--
Olive oil/blood	--	--	150

*Source: Adapted from Morgan et al., 1972; Sato and Nakajima, 1979; Powell, 1945

7.1.2. Absorption from the Gastrointestinal Tract. Absorption of CCl_4 from the gastrointestinal tract of dogs was studied by Robbins (1929). In a series of experiments, the author determined the amount of CCl_4 absorbed from the ligated stomach, small intestine and colon by measuring the concentration of CCl_4 exhaled. In all experiments, 3 mℓ of CCl_4 was administered. The greatest concentration of CCl_4 in exhaled air was seen after injection of the chemical into the small intestine. Direct injection of CCl_4 into the colon resulted in a lower concentration of the chemical in exhaled air. After introduction directly into the stomach by intubation, no CCl_4 was detected in exhaled air. The method of detection in these experiments was thermal conductivity, with stated detection limits of 1 part in 10 by volume of expired air. Thus, the results of the experiment can be viewed as a qualitative indication of relative absorption from the various components of the gastrointestinal tract, rather than as quantitatively accurate results.

The enhancement in the extent of CCl_4 absorption with ingestion of fat or alcohol has been reported (Nielsen and Larsen, 1965; Robbins, 1929; Moon, 1950). It also appears that the absorption of CCl_4 from the gastrointestinal tract may vary with different species because it occurs more quickly in rabbits than in dogs (Lamson et al., 1923).

Marchland et al. (1970) studied the effect of SKF 525A, the inhibitor of drug metabolizing enzymes, on the distribution of CCl_4 in rats. Prior to receiving 2 mℓ/kg $[^{14}C]CCl_4$, male Sprague-Dawley rats were administered either the SKF 525A (treated) or saline (control). The control animals were found to excrete at least 80% of the orally administered dose within 10 hours via the lungs. This indicates that at least 80% of the CCl_4 dose was absorbed orally.

Stokinger and Woodward (1958) report an "accurate" absorption factor of 50% via ingestion; however, they do not reference their conclusion.

7.1.3. **Absorption by Inhalation.** In 1950, von Oettingen et al. studied the absorption of CCl_4 by inhalation in beagle dogs. The sex was unspecified, but the authors stated that at least five dogs were used in each experiment. The dogs inhaled CCl_4 (purity unspecified) at a concentration of 94,500 mg/m^3 for 475 minutes through a two-way valve attached to the cannulated trachea. Blood samples were taken at unspecified intervals and analyzed for CCl_4. Data presented graphically showed that the concentration of CCl_4 in blood reached a maximum of 31.2-34.3 mg/100 cc (0.20-0.22 millimole %) after ~300 minutes of exposure and remained at that level for the duration of the exposure.

McCollister et al. (1951) investigated the absorption of CCl_4 by inhalation using Rhesus monkeys. Three female monkeys inhaled 99.9% [^{14}C]CCl_4 vapor at an average concentration of 290 mg/m^3 for 139, 344 or 300 minutes, respectively. By using the difference between inhaled and exhaled air, the authors calculated that the monkeys absorbed an average of 30.4% of the total amount of CCl_4 inhaled. Analysis of blood drawn after 270 minutes of exposure showed that the ^{14}C radioactivity was equal to 0.297 mg CCl_4/100 g of blood, distributed as follows: 56.2% as CCl_4, 16.5% as "acid-volatile" carbonates and 27.3% as nonvolatile material. No attempt was made to characterize metabolites in this study. The radioactivity no longer associated with the CCl_4 was described by the stage in the analytical procedure where it was found.

Stokinger and Woodward (1958) report an "accurate" absorption factor of 30% via inhalation; however, they do not reference their conclusion.

Lehmann and Schmidt-Kehl (1936) studied humans exposed to CCl_4 via inhalation. In separate experiments, individuals (number unknown) were enclosed in a room of CCl_4 vapors for varying amounts of time. The amount of CCl_4 available for inhalation was measured. The percent absorbed was calculated from the concentration of CCl_4 in the inhaled air minus the amount found in the exhaled breath. The reported range of percent absorption was 57-65%.

7.1.4. Absorption Through the Skin. McCollister et al. (1951) exposed the skins of one male and one female Rhesus monkey to $[^{14}C]CCl_4$ vapor. Blood and exhaled air were analyzed for ^{14}C radioactivity to determine the amount of absorption. After a skin exposure of 240 minutes to CCl_4 vapor at 3056 mg/m³, the blood of the female monkey contained CCl_4 at 0.012 mg/100 g and the exhaled air contained 0.0008 mg/ℓ. After exposure to 7345 mg/m³ for 270 minutes, blood of the male monkey contained CCl_4 at 0.03 mg/100 g and the exhaled air contained 0.003 mg/ℓ.

Three human volunteers, sex unspecified, immersed their thumbs in CCl_4 for 30 minutes in an experiment to measure skin absorption (Stewart and Dodd, 1964). The CCl_4 was analyzed by infrared spectroscopy and was found to contain no detectable impurities. The concentration of CCl_4 in alveolar air was used as the indicator of absorption and was measured at 10, 20 and 30 minutes after the start of exposure and at 10, 30, 60, 120 and 300 minutes after cessation of exposure. Carbon tetrachloride was present in the alveolar air at each time interval, reached a maximum concentration range of 2.8-5.7 mg/m³ 30 minutes after exposure and decreased exponentially thereafter. The authors concluded that CCl_4 could be absorbed by the skin in toxic amounts if the chemical came in contact with arms and hands.

7.2. DISTRIBUTION

The distribution of CCl_4 in humans, presumably after chronic low level exposure through various media (food, water and air), has been reported by McConnell et al. (1975). Measurements were made from postmortem tissues of several individuals; arithmetic means (in mg/kg) were approximately: body fat, 0.008 (range 0.002-0.024); liver, 0.003 (range 0.001-0.005); and kidney, 0.002 (range 0.001-0.003). Unfortunately these values represent possible concurrent trichloroethane concentrations.

Robbins (1929) administered 159 g (100 cc) CCl_4, purity unspecified, to three anesthetized dogs by stomach tube. The dogs were sacrificed at 6, 23 and 24 hours after treatment. Blood and various tissues were analyzed for CCl_4 by converting the organic chloride to inorganic chloride and titrating the inorganic chloride by the Volhard method, which is accurate to 0.1-0.2%. The results of the blood and tissue analysis are shown in Table 7-2. Note that the uptake of CCl_4 was probably reduced because of probable reduced G-I (portal) circulation during anesthesia.

From the experimental data, it appears the limit of detection was in the range of 4-5 mg CCl_4/100 g of tissue. In addition, it appears that the liver, bone marrow, blood and muscle retained the most CCl_4 for the longest time.

In 1950, von Oettingen et al. reported the tissue distribution of CCl_4 in beagle dogs, each weighing ~10 kg, exposed to CCl_4 in air at 94,500 mg/m³ for 475 minutes. The dogs were sacrificed at the end of the exposure. Tissue and blood samples were taken and analyzed for CCl_4. The concentration of CCl_4 (per 100 g of tissue) was 66 mg/100 g in the brain, 50 mg/100 g in the heart, 36 mg/100 g in the liver and 34 mg/100 g in the blood; the concentration in the fat was not determined. The investigators

TABLE 7-2

Carbon Tetrachloride Distribution at Various Times in Anesthetized Dogs After Administration by Stomach Tube (mg/100 g of tissue)*

Tissue	6 hours	23 hours	24 hours
Brain	--	17	9
Blood, portal	26	13	22
Blood, arterial	0	0	0
Bone marrow	--	66	--
Kidney	--	11	13
Liver	15	10	27
Lungs	--	trace	6
Muscle	--	trace	20
Pancreas	--	4.5	14
Spleen	--	5	--

*Source: Robbins, 1929

stated that the accumulation of CCl_4 in the brain was consistent with its high oil-water partition coefficient and resulted in its strong narcotic action.

McCollister et al. (1951) reported the tissue distribution of CCl_4 in a Rhesus monkey exposed to 290 mg/m³ of $[^{14}C]CCl_4$ for 300 minutes. The tissue distribution, as calculated from the ^{14}C radioactivity, is shown in Table 7-3. The concentration of CCl_4 was greatest in the fat, followed by the liver and bone marrow.

Fowler (1969) studied the distribution of CCl_4 in the tissues of rabbits given the chemical by stomach tube. Five rabbits were given CCl_4 (1 mℓ/kg bw) as a 20% (v/v) solution in olive oil. Analysis of the CCl_4 by GC showed $\leq$0.125 mg/kg hexachloroethane. The rabbits were sacrificed 6, 24 and 48 hours after treatment and the tissues analyzed for CCl_4 by GC/ECD. Six hours after CCl_4 was administered, the tissue concentrations (per kg of tissue) were 787±289 mg/kg (standard error) in fat, 96±11 mg/kg in liver, 21±12 mg/kg in muscle and 20±13 mg/kg in kidney. By 48 hours, these concentrations had dropped to 45±12 mg/kg in fat, 4±0.1 mg/kg in liver and 0.5±0.3 mg/kg in kidney and muscles.

It is difficult to compare these four distribution studies, because species, sacrifice times, doses and routes of exposure varied. Moreover, not all important tissues were sampled [e.g., von Oettingen, et al. (1950) did not sample the fat]; and the number of animals in two cases was small [e.g., Robbins (1929) reported results on three dogs; McCollister et al. (1951) reported results on one monkey]. Nonetheless, it appears that the concentration of CCl_4 will be highest in fat, liver, bone marrow, blood and perhaps kidney or brain after administration by either oral or inhalation routes. Certainly more work could be done in this area.

TABLE 7-3

Tissue Distribution of [^{14}C] Carbon Tetrachloride Inhaled by a Female Rhesus Monkey*

Tissue	Carbon Tetrachloride (mg/100 g of tissue)
Fat	2.46
Liver	0.94
Bone marrow	0.93
Blood	0.31
Brain	0.30
Kidney	0.23
Heart	0.14
Spleen	0.10
Muscle	0.06
Lung	0.04
Bone	0.04

*Source: McCollister et al., 1951

7.3. METABOLISM

Chloroform was one of the first CCl_4 metabolites to be described (Butler, 1961). Eight dogs were exposed to CCl_4 by tracheal cannula at the rate of 8000 mg/hr into inhaled air for 3 hours. At the cessation of exposure, the exhaled air from the dogs was collected and analyzed by both gas chromatography and the Fujiwara reaction, a colorimetric procedure for the identification of chloroform. Chloroform was detected in the exhaled breath by both of these methods. The total amount of chloroform exhaled in 2 hours by each dog was estimated at 0.1-0.5 mg by GC analysis. Tissue homogenates were also shown to metabolize CCl_4 to chloroform.

Evidence of metabolism to a free radical was suggested by studies showing hexachloroethane to be a CCl_4 metabolite (Bini et al., 1975). Five Wistar rats were administered 160-800 mg CCl_4 dissolved in liquid paraffin by gavage following a 24-hour fast. The animals were sacrificed 15 minutes to 8 hours after treatment. A graph displaying CCl_4 concentrations in rat liver versus time showed the chemical at ~0.9 mg/kg of tissue after 15 minutes and at maximal concentration (1.7 mg/kg) after 120 minutes. Gas chromatography analysis showed that chloroform was maximal at 0.037 mg/kg after 15 minutes; after 4 hours it had declined to 0.007 mg/kg. Hexachloroethane was also present after 4 hours at 0.005 mg/kg. The authors explained the formation of both chloroform and hexachloroethane as CCl_4 metabolites by proposing that the trichloromethyl free radical was the primary metabolite of CCl_4.

^{14}C-labeled carbon dioxide ($[^{14}C]CO_2$) was detected in the exhaled air of Rhesus monkeys after a 344-minute exposure to $[^{14}C]CCl_4$ at 290 mg/m^3 by inhalation (McCollister et al., 1951). The amount of $[^{14}C]CO_2$ exhaled during the 7-day period following exposure was reported to be 10-20% of the total radioactivity expired. The authors fitted these data to a straight line, integrated the resulting equation from 18-1800 hours (75 days) and estimated that 4.4 mg or 11% of the total amount of radioactivity eliminated was excreted as CO_2.

Shah et al. (1979) studied the metabolism of $[^{14}C]CCl_4$ by rat liver _in vitro_. Samples of liver homogenate equivalent to 0.167 g of tissue were incubated for 30 minutes at 37.5°C with 10 μmole of $[^{14}C]CCl_4$ alone, and with either NADH or NADPH or both. $[^{14}C]$carbon dioxide was detected by scintillation counting. The results are shown in Table 7-4. The addition of NADPH or NADH, separately or as a mixture, appeared to result in substantial conversion of CCl_4 to CO_2.

Shah et al. (1979) tested for the possible formation of carbonyl chloride in hepatic CCl_4 metabolism by adding L-cysteine to the _in vitro_ rat liver system described above. Carbonyl chloride and L-crysteine are known to react chemically to form a condensation product, 2-oxothiazolidine-4-carboxylic acid. The presence of the condensation product was confirmed by thin-layer chromatography (TLC) and mass spectrometry (MS). The authors inferred from the presence of 2-oxothiazolidine-4-carboxylic acid that carbonyl chloride was formed in the metabolism of CCl_4 by rat liver microsomes. The authors postulated a mechanism of biotransformation for CCl_4 which involved a sequential oxidation of CCl_4 while bound to a heme (Figure 7-1). Release of bound intermediates then gave rise to different metabolites at the site of release.

Fowler (1969) detected hexachloroethane and chloroform in the tissues of rabbits administered CCl_4 orally. As previously described, a total of 15 rabbits were given CCl_4 at 1 mℓ/kg bw and sacrificed in groups of five at 6, 24 and 48 hours after exposure. Samples of fat, liver, kidney and

TABLE 7-4

Conversion of [^{14}C]Carbon Tetrachloride to [^{14}C]Carbon Dioxide by Rat Liver Homogenate*

Nucleotide Added	[^{14}C]CO_2 (nmole/g liver, mean ± standard deviation)
None	27 ± 5
NADH	373 ± 17
NADPH	464 ± 33
NADH + NADPH	472 ± 21

*Source: Shah et al., 1979

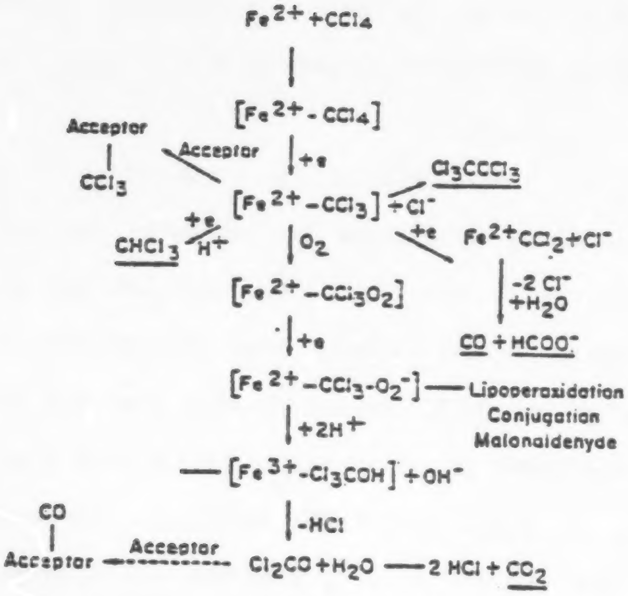


FIGURE 7-1

Pathways of Carbon Tetrachloride Metabolism. Products identified as carbon tetrachloride metabolites are underlined. The electrons utilized in the reactions are assumed to come from NADH or NADPH via the flavoprotein cytochrome reductases. Fe^{2+} and Fe^{3+} denote the respective ferro- and ferricytochromes.

Source: Redrawn from Shah et al., 1979

muscle tissue were analyzed for chloroform and hexachloroethane by gas chromatography. The results of the analysis are shown in Table 7-5. The fat contained the highest amounts of hexachloroethane at each sampling time, but the highest concentrations of chloroform appeared in the liver.

For a complete discussion on metabolic pathways hypothesized to be mechanisms of toxicity, see Chapter 8, Section 8.3.

7.4. ELIMINATION

McCollister et al. (1951) studied the elimination of [^{14}C]CCl$_4$ from Rhesus monkeys exposed by inhalation at 290 mg/m^3 for 344 minutes. The total ^{14}C radioactivity in the blood decreased 12% during the first 10 minutes after exposure. Graphs of data from the analysis of blood samples obtained periodically for 10-12 days following exposure showed that the level of CCl$_4$ in the blood decreased exponentially with time. At 10 days, the level of CCl$_4$ in blood was ~0.009 mg/100 g. The authors estimated that 21% of the total amount of CCl$_4$ absorbed was eliminated in expired air during the first 18 days. By extrapolation of these data, the authors concluded that after 1800 hours (75 days), ~51% of the CCl$_4$ initially absorbed would be eliminated in exhaled breath either as CCl$_4$ or CO$_2$. Analysis of urine and feces showed measurable amounts of radioactivity after 15 and 12 days, respectively. The authors interpreted these findings as an indication that significant quantities of CCl$_4$ and/or metabolites may be excreted by these routes.

7.5. SUMMARY

Carbon tetrachloride is readily absorbed from the lungs and the gastrointestinal tract, as expected from its partition coefficients. Although few quantitative data are available on the amount of CCl$_4$ absorbed through the

TABLE 7-5

Chloroform and Hexachloroethane in Tissues of Rabbits Given Carbon Tetrachloride Orally*

Sample Time	Tissue	$CHCl_3$ (µg/g tissue)	Cl_3CCCl_3 (µg/g tissue)
6 hours	fat	4.7 ± 0.5	4.1 ± 1.2
	liver	4.9 ± 1.5	1.6 ± 0.5
	kidney	1.4 ± 0.6	0.7 ± 0.2
	muscle	0.1 ± 0.1	0.3 ± 0.2
24 hours	fat	1.0 ± 0.2	16.5 ± 1.6
	liver	1.0 ± 0.4	4.2 ± 1.8
	kidney	0.4 ± 0.2	2.2 ± 1.1
	muscle	0.1 ± 0.1	0.5 ± 0.2
48 hours	fat	0.4 ± 0.1	6.8 ± 2.4
	liver	0.8 ± 0.2	1.0 ± 0.3
	kidney	0.2 ± 0	trace
	muscle	0.1 ± 0.1	trace

*Source: Fowler, 1969

lungs, the chemical and its metabolites have been reported in blood, many tissues, exhaled air, urine and feces after administration by this route. Carbon tetrachloride is also absorbed through the skin.

The best available data concerning CCl_4 absorption come from two studies and one report:

- 30.4% via inhalation in monkeys (McCollister et al., 1951)
- 57-65% via inhalation in humans (Lehmann and Schmidt-Kehl, 1936)
- 30% via inhalation and 50% via ingestion (Stokinger and Woodward, 1958).

The Stokinger and Woodward report has been criticized for not including substantiating information or alternatively, citing the specific literature. There are some considerations associated with extrapolating from animal data to humans in that animals may alter their breathing patterns during an experiment or may not be good models insofar as their metabolism is concerned. This is not to say that the animal data or Stokinger and Woodward report should be overlooked. Therefore, it is recommended that a 40% absorption coefficient be used when the route of exposure is via inhalation and that a 100% absorption coefficient be used when the route of exposure is via ingestion. The former is a compromise based upon the available information and the latter is the conservative estimate due to little information.

In the studies reviewed, CCl_4 appears to be distributed to all major organs following absorption. The highest concentrations have been found in the fat, liver, bone marrow, blood, brain and kidney.

Carbon tetrachloride metabolism has been reported to occur primarily in the liver. Carbon tetrachloride has been postulated to be metabolized to a trichloromethyl radical bound to an iron atom in the cytochrome heme moiety.

This trichloromethyl radical is thought to be either further metabolized or released as a free radical. It is suggested that the trichloromethyl free radical can undergo a variety of reactions, including macromolecular binding, hydrogen abstraction to form chloroform, and dimerization to form hexachloroethane.

Carbon tetrachloride and its metabolites have been reported in many studies to be excreted primarily in exhaled air, but also in the urine and feces. However, pharmocokinetic data on these processes are apparently lacking.

8. TOXICOLOGY: ACUTE, SUBCHRONIC AND CHRONIC

Hepatotoxicity is the major effect reported to be produced by acute exposure of animals to CCl_4. Hepatic necrosis and fatty liver degeneration have been documented after both inhalation exposure and ingestion. Hepatotoxicity has sometimes been accompanied by toxic effects to the kidney and lungs. In addition, prenatal toxic effects have been demonstrated after inhalation of the chemical by pregnant rats. This chapter discusses the effects from acute exposure (single dose, 1 day or several days defined in rodents), subchronic exposure (2 weeks to somewhat more than 90 days defined in rodents) and chronic exposure ($\geq$6 months defined in rodents) to CCl_4. Emphasis is placed on studies in which dose/response relationships have been developed. Toxic responses occurring prior to carcinogenicity are reported in Chapter 11.

8.1. EXPERIMENTAL ANIMALS

8.1.1. **Acute.** The toxicity from acute exposure to CCl_4 has been documented extensively. Because defining the dose range that produces minimal health effects is an objective of this report, this section will concentrate on those studies that (1) describe nonlethal effects and (2) provide data on a range of doses from which dose-response relationships can be determined. For this reason a number of studies referring to LD_{50}s will not be discussed. Table 8-1 summarizes some of the lethal dose data reported for CCl_4 in various species.

Administration of CCl_4 results in a number of acute systemic effects. Its hepatic effects are the most pronounced; CCl_4 causes necrosis and fatty liver degeneration. These effects have been well documented in many scientific studies. However, toxicity to other organs has also been described. The following subsections will focus on such organ toxicity.

TABLE 8-1

Toxic Doses and Effects of Carbon Tetrachloride in Animals[a]

Animal	Route of Administration	Effect[b]	Dose
Rat	oral	LD_{50}	2,800 mg/kg
Mouse		LD_{50}	12,800 mg/kg
Dog		LD_{Lo}	1,000 mg/kg
Rabbit		LD_{50}	6,380 mg/kg
Rat	intraperitoneal	LD_{50}	1,500 mg/kg
Mouse		LD_{50}	4,675 mg/kg
Dog		LD_{Lo}	1,500 mg/kg
Rabbit		LD_{Lo}	478 mg/kg
Rat	inhalation	LC_{50}	624.80 mg/m^3
Mouse		LC_{50}	1,487.97 mg/m^3
Cat		LC_{Lo}	5,952.83 mg/m^3
Guinea pig		LC_{Lo}	3,124.02 mg/m^3
Cat	subcutaneous	LD_{Lo}	300 mg/kg
Rabbit		LD_{Lo}	3,000 mg/kg

[a] Source: U.S. EPA 1981

[b] LD_{50}, dose lethal for 50% of animals
LC_{50}, concentration lethal for 50% of animals
LD_{Lo}, lowest lethal dose
LC_{Lo}, lowest lethal concentration

8.1.1.1. LIVER EFFECTS -- Functional changes in mouse liver as a result of CCl_4 exposure were measured by increases in activity of the enzyme serum glutamic-pyruvic transaminase (SGPT) and in sulfobromophthalein (BSP) retention (Klaassen and Plaa, 1966). Male Swiss-Webster mice were administered various amounts of analytical grade CCl_4 i.p. in corn oil in a final volume of 10 mℓ/kg bw. Mice treated only with corn oil were used to establish the normal range of values for BSP retention and SGPT activity, which were determined 24 hours after treatment. The authors reported the median effective doses of CCl_4 as 15.9 mg/kg bw for elevation of SGPT activity and 94 mg/kg bw for BSP retention. The authors did not report the range of CCl_4 doses used or the number of animals used at each dose.

In an additional study, Klaassen and Plaa (1967) further defined a dose-response relationship for CCl_4 exposure and elevated SGPT levels in mice. They used the "up and down" method in which one dose (given in Table 8-2) of the compound was given intraperitoneally; the animal's SGPT activity 24 hours after the dose was noted. If the enzyme activity was elevated, the dose was decreased 40% and the experiment repeated in another animal. If no effect was noted, the dose was increased 40% and the experiment repeated in another animal. This series was repeated three times after one positive and one negative response had been obtained. The results for mice are shown in Table 8-2. The authors concluded that 13 mg/kg bw was the median effective dose (ED_{50}) of CCl_4 in mice as measured by elevated SGPT values.

Sein and Chu (1979) studied the effect of CCl_4 on the level of the liver enzyme glucose-6-phosphatase in mice. Groups of six male LAC strain mice were treated i.p. with 795, 1590 or 3180 mg/kg bw CCl_4 (purity unspecified) in paraffin oil. The animals were sacrificed 24 hours after treatment. Control animals (number unspecified) were given paraffin oil and

TABLE 8-2

SGPT Values of Mice Administered Carbon Tetrachloride Intraperitoneally in "Up and Down" Experiment[a]

Animal	Dose (mg/kg bw)	Response[b]
1	17.5	E
2	13	N
3	17.5	E
4	13	E
5	8	N
ED_{50}	13	

[a] Source: Klaassen and Plaa, 1967

[b] E = Elevated SGPT after 24 hours
N = Normal SGPT after 24 hours

ED_{50} = Median effective dose

sacrificed on the same schedule. The livers were removed and analyzed for glucose-6-phosphatase. The results of the analysis showed that after treatment with CCl_4 at 795 or 1590 mg/kg, the enzyme level fell to 40% of the control value. At a dose of 3180 mg/kg, the enzyme level had decreased to 20% of the control value.

A series of experiments to determine the effects of single CCl_4 exposures on rats were performed by Murphy and Malley (1969). Adult male Holtzman rats (250-350 g) were administered various doses of undiluted CCl_4 by gavage. Control animals were administered equal volumes of water. At 2-20 hours after treatment, animals were sacrificed and liver enzyme activities and liver weights were measured. The results are shown in Table 8-3. The animals receiving CCl_4 at 1600 mg/kg bw were sacrificed 20 hours after treatment and the livers examined histopathologically. The examination showed extensive fatty infiltration, inflammation and some centrilobular necrosis. The liver-to-body weight ratios were also increased.

The effect of single exposures on the activities of the corticosterone-inducible liver enzymes tryptophan pyrrolase and tyrosine-ketoglutarate transaminase was studied. Groups of rats (4-6 in each group, 8 untreated controls) were treated with CCl_4 (0, 400, 800 and 1600 mg/kg bw by gavage) and sacrificed 5 hours after treatment. Data graphs showed that the enzyme levels were increased roughly in proportion to the dose.

Similar studies on the effect of CCl_4 administration on serum activity of liver enzymes in rats were performed by Drotman and Lawhorn (1978). Groups of four male Cox rats were administered CCl_4 i.p. at 60, 120, 240 or 480 mg/kg bw in a total volume of 1 mℓ in corn oil and exsanguinated at specified time intervals. Serum activities were determined for the enzymes

TABLE B-3

Effects of Oral Carbon Tetrachloride on Liver Weight and Liver and Plasma Enzyme Activities in Male Rats[a]

Dose (mg/kg bw)	Time (hr)	Number of Animals	Plasma AKT[b]	Liver[c]			
				AKT[b]	TKT[b]	AP[b]	Weight (g/100g bw)
0	-	7	2.6 ± 0.3[c]	2360 ± 182	93 ± 11	14.9 ± 1.0	2.75 ± 0.06
3200	2	4	2.1 ± 0.2	-	170 ± 12	14.5 ± 1.0	2.94 ± 0.04
3200	5	5	13.2 ± 3.4	1813 ± 331	330 ± 36	14.2 ± 1.3	3.26 ± 0.10
3200	20	5	35.2 ± 4.2	1174 ± 559	361 ± 42	34.6 ± 5.8	4.36 ± 0.06
2400	20	4	35.2 ± 3.4	1585 ± 148	305 ± 21	37.1 ± 1.6	3.95 ± 0.07
1600	20	5	18.1 ± 4.3	1596 ± 194	294 ± 61	33.3 ± 3.6	3.90 ± 0.05
800	20	4	9.3 ± 2.3	2120 ± 182	138 ± 5	29.1 ± 6.5	3.47 ± 0.07

[a] Source: Adapted from Murphy and Malley, 1969

[b] AKT = alanine-α-ketoglutarate transaminase
TKT = tyrosine-α-ketoglutarate transaminase
AP = alkaline phosphatase

[c] means ± SE in micromoles of product formed per gram of fresh liver or milliliter of plasma per hour

sorbitol dehydrogenase (SSDH), ornithine carbamyl transferase (SOCT), aspartate aminotransferase (SAST) and isocitric dehydrogenase (SICDH). Liver specimens were taken from each animal and scored for histopathological changes. The results of the enzyme analyses and histopathology are tabulated in Table 8-4 by dose and hours after dose. The SOCT activities showed the best correlation with liver histopathology in time of appearance as well as extent of damage. The authors concluded that SOCT levels were a sensitive indicator of liver damage.

Effects of acute exposure to low levels of CCl_4 were also reported by Korsrud et al. (1972). Male Wistar rats (260-400 g; 8-10 animals per treatment group) were administered single oral doses of CCl_4 (0-4000 mg/kg bw) in corn oil (5 mℓ/kg bw). The rats were fasted for 6 hours before treatment and for 18 hours afterward, and then sacrificed. Assays included liver weight and fat content, serum urea and arginine levels, and levels of nine serum enzymes produced mainly in the liver. At 20 mg/kg bw there was histopathologic evidence of toxic effects on the liver. These changes included a loss of basophilic stippling, a few swollen cells and minimal cytoplasmic vacuolation. At 40 mg/kg bw, liver fat, liver weight, serum urea and levels of five of the nine liver enzymes were increased while serum arginine decreased. At higher doses the remaining four enzyme levels were also elevated.

In addition to the study on hepatic effects of CCl_4 in mice described earlier in this section, Klaassen and Plaa (1967) also investigated the hepatic effects of CCl_4 exposure on dogs. Male and female mongrel dogs were treated i.p. with CCl_4 at 22-38 mg/kg bw in an "up and down" experimental design. Blood samples were taken for measurement of SGPT 24 hours after administration. Control dogs had serum SGPT activity of 36±7 units.

TABLE 8-4

Effects of Carbon Tetrachloride on Liver Histopathology and Serum Enzyme Levels[a]

Dose (mg/kg)	Hours After Dose	Histology[b]	Serum Enzyme Concentrations Relative to Pretreatment Levels			
			SOCT	SSDH	SAST	SICDH
60	0	0	1.0	1.0	1.0	1.0
	6	2	9.6*	2.5	2.0	1.4
	12	1	8.2*	4.4*	2.5*	1.1
	24	1	5.7*	1.7	2.0	1.3
	36	0	1.0	1.0	1.0	1.0
120	0	0	1.0	1.0	1.0	1.0
	24	3	14.0*	7.2*	2.1*	1.1
	48	2	7.4*	1.0	1.0	1.0
	96	1	1.8	1.7	1.3	1.3
	168	0	1.0	1.0	1.0	1.0
240	0	0	1.0	1.0	1.0	1.0
	24	3	31.0*	17.4*	5.8*	7.2*
	48	4	180.0*	43.4*	17.0*	7.4
	96	1	6.6*	4.7*	3.6*	2.0
	168	0	1.1	1.0	1.9	2.0
480	0	0	1.0	1.0	1.0	1.0
	24	3	28.4*	90.0*	6.1*	5.4*
	48	4	465.5*	163.5*	18.4*	50.4*
	96	1	1.0	8.4*	1.8*	2.0
	168	0	1.0	1.4	1.0	2.1

[a] Source: Adapted from Drotman and Lawhorn, 1978

[b] 0 = No observable changes.
1 = Minimal changes. Large central vein, swelling of hepatocyte, etc.
2 = Mild degenerative change. Loss of cord arrangement.
3 = Moderate degenerative change. Pale cytoplasm, spindle cell.
4 = Marked degenerative change. Centrilobular fatty degeneration.

*Significantly different from zero time as determined by one-way analysis of variance of the log-transformed data ($p \leq 0.01$).

Therefore, 36+2 standard deviations or 50 units was chosen as the upper limit of the normal value. The results of the analysis are shown in Table 8-5. The SGPT returned to normal in 17-18 days. Animals were then sacrificed and the livers were examined histopathologically. They showed moderate vacuolation of the centrilobular and midzonal hepatocytes as well as traces of brown material in the cytoplasm of centrilobular Kupffer cells.

Gardner et al. (1924) also studied the acute toxic effects from ingestion of CCl_4 in dogs. Effects ranged from no apparent effect at 0.01 mℓ/kg (15.89 mg/kg) to centrilobular necrosis at 0.05 mℓ/kg (79.45 mg/kg). Rabbits similarly treated experienced liver necrosis at 0.1 mℓ/kg (158.9 mg/kg).

Kronevi et al. (1979) administered CCl_4 epicutaneously to guinea pigs. Dermal effects were seen (see Section 8.1.1.4.) along with effects on the liver. Liver morphology was characterized by hydropic and necrotic changes. Altered liver morphology was seen after 16 hours when hepatocytes in the central two-thirds of each lobule showed marked hydropic changes which were characterized by large, clear cytoplasmic spaces. It was noted there was also a tendency toward necrotic lesions characterized by homogeneous, slightly eosinophilic and slightly PAS-positive structures within the cytoplasm. Glycogen was absent and the nuclei showed a tendency to degeneration.

In studying the renal effects of CCl_4 in cats (see Section 8.1.1.2.), Wong and DiStefano (1966) noted a delayed liver reaction. Liver weights were significantly increased 24 hours following inhalation exposure.

8.1.1.2. KIDNEY EFFECTS -- In experiments conducted by Plaa and Larson (1965) using CCl_4, high doses did not induce renal failure as measured by phenolsulfonphthalein (PSP) excretion in mice although pathological kidney alterations were present. Male Swiss mice (18-30 g) were

TABLE 8-5

SGPT Activity in Dogs 24 Hours After Intraperitoneal Administration of Carbon Tetrachloride in "Up and Down" Experiment[a]

Animal	Dose (mg/kg)	Response[b]
1	22.2	N
2	30.2	E
3	22.2	N
4	30.2	N
5	38	E
ED_{50}	32	

[a] Source: Adapted from Klaassen and Plaa, 1967

[b] N = normal SGPT after 24 hours
E = elevated SGPT after 24 hours

ED_{50} = Median effective dose

given i.p. injections of CCl_4 (1600-6400 mg/kg bw) dissolved in corn oil at a final amount of 10 mg/kg bw. The animals were then hydrated with tap water (50 mg/kg bw) by gavage and placed on a urinary collection unit for 2 hours. Even CCl_4 doses lethal in some animals ($\geq$6400 mg/kg bw) failed to cause renal dysfunction in the majority of survivors, as measured by excretion of PSP, urinary protein and glucose. At a high nonlethal dose (3260 mg/kg bw), minimal renal dysfunction was observed after 96 hours. Histologic examination of kidney sections from five mice administered this dose indicated that one of the mice had necrosis of proximal convoluted tubules, and four of five mice had swelling of the tubules.

Carbon tetrachloride did, however, decrease activity of glucose-6-phosphatase in the kidney (Sein and Chu, 1979). Male mice (40-50 days old, weighing 24-28 g) were injected i.p. with CCl_4 at 795, 1590 or 3180 mg/kg bw in paraffin oil. Twenty-four hours after injection of 795 or 1590 mg/kg bw, the kidney glucose-6-phosphatase activity decreased to 77 or 65% of the control value, respectively. Increasing the dose to 3180 mg/kg bw had no further effect on the kidney enzyme level.

These results were in contrast to the liver glucose-6-phosphatase level discussed earlier, which decreased to 40% of the control value at the two lower doses and decreased further to 20% of the control value at the 3180 mg/kg bw dose. The authors attributed these differences to the limited metabolic capacity of the kidneys.

Klaassen and Plaa (1967) studied the effect of CCl_4 exposure on kidney function in dogs. PSP excretion of <39% of control value was considered indicative of renal dysfunction. An unspecified number of male and female mongrel dogs were treated i.p. with CCl_4 at 22-38 mg/kg bw, and the 24-hour excretion rate for PSP was determined. Control dogs were used to determine a normal range for PSP excretion. None of the dogs treated with CCl_4 exhibited decreased PSP excretion. However, on histopathic examination of the kidneys from the treated dogs, the Bowman's capsules appeared dilated with some contraction of glomerular tufts and calcification of a small number of tubules in the medulla.

Striker et al. (1968) examined the structural and functional changes in the rat kidney during CCl_4 intoxication. Exposure was 2.5 mℓ/kg bw in an equal volume of mineral oil by gastric intubation. A progressive increase in the size and paleness of the kidney was apparent during the first 2 days after exposure, with maximum effects seen at 48 hours. Alterations were limited to the proximal tubule and appeared to involve primarily the middle and lower segments. The alterations seen were sequential and reversible. The earliest morphological change was in the mitochondria, followed by cellular swelling manifested by loss of basilar interdigitations and swollen microvilli. Occurring later was the appearance of large aggregates of smooth-surfaced membranous profiles. By 5 days after exposure, all alterations were reversed.

Also apparent were differences in serum parameters reflecting kidney function. Serum creatinine and blood urea nitrogen peaked at 12 hours and 24 hours, respectively. Total serum bilirubin was elevated 12-48 hours. By 5 days all values returned to normal (Striker et al., 1968).

Wong and DiStefano (1966) examined lipid accumulation and histologic changes in the kidney of the cat following inhalation of 10,000 ppm CCl_4 for periods of 15, 30, 60 and 240 minutes. Kidney weight:body weight ratios increased significantly following 60- and 240-minute exposures. The renal cortical lipid content increased significantly within 15 minutes and an accumulation of fat droplets was seen histologically in 30-60 minutes. One hour after the termination of CCl_4 inhalation, the kidneys were enlarged and the weight continued to increase past the 24-hour observation period.

8.1.1.3. LUNG EFFECTS -- Boyd et al. (1980) investigated the effect of ingestion and inhalation of CCl_4 on pulmonary Clara cells in Swiss mice. For the ingestion study, the mice were treated with CCl_4 (4000 mg/kg bw) in a 50% sesame oil solution and sacrificed 16 hours after treatment. The lungs were removed and examined by electron microscopy. The examination showed the Clara cells to have massive dilation of vesicles of smooth endoplasmic reticulum, increased mitochondrial staining density, ribosomal disaggregation, nuclear condensation and occasional cellular necrosis. Additional experiments with oral CCl_4 doses of <1600 mg/kg bw did not produce any pulmonary lesions visible by light microscopy. Doses of 2400-4800 mg/kg bw produced Clara cell lesions similar on electron microscopic examination to those previously described. The extent of damage was proportional to the dose administered. In addition, the time course of the Clara cell damage caused by ingestion of CCl_4 (4000 mg/kg bw) was studied. Pulmonary tissue was evaluated by light microscopy at 12, 24, 36, 48, 96 and 168 hours. The lesions were present at 12 hours, maximal at 24 hours, and less intense at 36 hours. By 48 hours, the lesions were seen infrequently, and at 96 and 168 hours the pulmonary bronchioles appeared normal.

The pulmonary toxicity of inhaled CCl_4 was also studied by Boyd et al. (1980). Swiss mice were exposed to CCl_4 vapor at 71,800, 144,000, 287,000 or 574,000 mg/m^3 for 60, 60, 12 or 2 minutes, respectively. The animals were sacrificed 24 hours after exposure, and the lungs were examined. Marked Clara cell lesions similar to those seen after oral exposure were seen at all exposure levels; necrosis was reported to be more frequent after inhalation than after oral exposure, but no effort to quantify this finding was reported.

Gould and Smuckler (1971) detailed the structural alterations in rat lungs following CCl_4 ingestion. Male Sprague-Dawley rats (200-250 g) were fasted 16 hours prior to administration of CCl_4 (4000 mg/kg bw) by gavage. The animals exhibited piloerection and lassitude 3-4 hours after treatment. Necropsies were performed on all animals. Microscopic examination of the lungs of treated rats revealed perivascular edema and mononuclear infiltration in the first 4 hours after treatment. These areas were local but were estimated to involve 10% of the parenchyma. Areas of atelectasis and intra-alveolar hemorrhage involving 15-20% of the parenchyma were observed 8-12 hours after treatment.

Electron micrographs of rat lungs after CCl_4 ingestion showed granular pneumocytes containing swollen inclusions with decreased osmiophilia and attenuated lameliae 1 hour after treatment. These changes were more severe 4 hours after treatment. By 4-8 hours after treatment, cytoplasmic edema, dislocation of dense ribosomal aggregates and mitochondrial disruption were apparent. Multivesicular bodies were "conspicuously decreased" within the granular pneumocytes. Necrosis was evident 12-24 hours after treatment. One hour after administration, endothelial cells displayed markedly increased pinocytotic vesicles. Severe disruption of endothelial cells was evident from 8 hours onward. Ultrastructural damage was seen in all components of the alveolar wall, and fibrin was observed within alveoli. The authors interpreted these findings as showing significant alterations in vascular permeability.

Lesions of the Clara cells in the lungs of male Sprague-Dawley rats orally treated with CCl_4 were observed by Boyd et al. (1980). The CCl_4 was administered by gavage at doses of 3816, 5088 and 7155 mg/kg as a 50% solution in sesame oil. Control animals received sesame oil only. Clara cell lesions occurred at the two highest doses. The authors stated that the lesions were less pronounced than those in mice exposed to comparable amounts of CCl_4.

Chen et al. (1977) examined the lung effects of rats given a single CCl_4 exposure by gastric intubation or by inhalation. Exposures were: 2.5 mℓ/kg bw by gastric intubation, and 30 minutes in air containing 4.38% CCl_4 (280,400 mg/m³) by inhalation. Both the orally administered and inhaled CCl_4 markedly modified the lung and liver cytochrome P-450 content, but there was a greater response in the pulmonary tissue. Inhalation resulted in a less significant depression of activity in both organs (Chen et al., 1977). Morphologic analyses of the lung revealed focal changes by 1-7 days in pulmonary architecture consisting of areas of alveolar collapse, septal thickening, transudation and modification of type II pneumonocytes.

8.1.1.4. DERMAL EFFECTS -- Kronevi et al. (1979) examined the effects on guinea pigs following epicutaneous administration of 1 mℓ CCl_4. Slight karyolysis was seen. After 15 minutes, marked spongiosis developed. Fifteen minutes and onward, karyopyknosis was evident. The authors saw progressing nuclear pyknosis and junctional separation between the basement membrane and the basal cells along with induced spongiosis appearing before the junctional separation attributed to the CCl_4 exposure.

8.1.1.5. BIOCHEMICAL AND OTHER EFFECTS -- Merkur'eva et al. (1979) studied the effects of continuous inhalation of CCl_4 on the rat enzyme system. Three stages of pathological changes in the liver were defined as characterized by morphological and biochemical parallels in the development of effects. Random-bred, sexually mature male albino rats (strain not given), totaling 55, were exposed to 300 mg/m³ CCl_4. The exposure resulted in a 45% reduction in the activity of N-acetyl-β-d-glucosaminidase

24 hours following initiation, and an overall reduction in the activity of the same enzyme by the 14th day of the experiment. The activity of serum hyaluronidase increased to a maximum 3 days into the experiment with the average increase being 96%. After 3 days, a gradual decrease was observed. However, at 14 days, levels were still increased over controls by 68%.

The peak in activity of serum hyaluronidase was accompanied by an increase in the activity of acid phosphatase (by 38%). In addition, the activities of ßglucosidase and ß-galactosidase decreased to 62% and 73%, respectively, at 8 days. Fourteen days following initiation, a 3-fold increase in the activity of ß-glucosidase was observed along with a decrease (by 30%) in the activity of acid phosphatase. Finally, a 39% inhibition of N-acetyl-neuraminic acid aldolase was observed.

Following injection of CCl_4, Wyrebowska and Jerzykowski (1980) saw a change in enzyme activity in rat serum. Mature male and young Wistar rats were given a single 2 mℓ/kg bw dose of CCl_4 i.p. dissolved in sterile vegetable oil (1:1) or 1 mℓ/kg CCl_4. Following this dose, both the alkaline and acid forms of aminopropanol dehydrogenase appeared in the blood serum. Maximum activity occurred 24 hours after administration in the young rats and 12 hours after administration in the mature male rats.

Mikhail et al. (1978) injected male and female adult rats intraperitoneally with 0.5 mℓ of a 1:1 mixture of CCl_4 in mineral oil per 100 g bw (0.005 mℓ of the mixture per g bw). This resulted in an increase in serum iron, copper, zinc, calcium, potassium and sodium 24 hours after administration. There was no change observed in serum magnesium. The authors attribute the rise in some serum chemistries to the known hepatotoxic effect of CCl_4. They conclude that the disturbance in minerals metabolism is one of the earliest lesions in CCl_4 poisoning (Mikhail et al., 1978).

David et al. (1981) examined the effect of different exposure schemes upon biochemical and morphological changes in the rat liver. Groups of six male Wistar rats, 7 months old, were exposed to CCl_4 vapor under different exposure schedules. Schedules were designed in such a way to expose each group to a constant product of concentration and time (CT = 1950 mg/m³) for 4 successive days a week.

It was found that a higher dose given over a shorter period of time had a greater effect than a lower dose given over a longer period of time (1625 mg/m³ for 72 minutes vs. 325 mg/m³ for 6 hours). Also, continuous exposure (18 minutes) to 6500 mg/m³ produced a greater effect than intermittent exposure (3 minutes, 6 times with 1 hour intervals) to 6500 mg/m³. The authors conclude that sensitivity of the liver is more influenced by the concentration of CCl_4 in the inhaled air than in the total amount inhaled, the theory being that the former allows more CCl_4 into the blood entering the liver. As the authors explain, this information is important in putting time weighted averages (TWAs) in the workplace.

8.1.2. Subchronic. The toxicity followng subchronic exposure to CCl_4 has not been as extensively described as the toxicity following acute exposure. Paquet and Kamphausen (1975) examined biochemical changes in female Wistar rats following subchronic exposure to CCl_4. Administration of CCl_4 was by subcutaneous injection of 1 mℓ/kg bw in an equal amount of peanut oil at 7-day intervals for 8 weeks. The authors described the changes in stages. In stage 1 there was a decrease in pseudocholinesterasis indicative of the stage of liver necrosis. In stage 2, the triglycerides reached a high plateau; there was an increase in SGOT, an increase in BSP retention and a continued decrease in pseudocholinesterasis. The second stage was characterized by massive fatty infiltration of the liver and increased necrosis with liver fibrosis at the end. In stage 3, SGOT continued to increase along with hydroxyproline, triglycerides and BSP retention. This final stage was characterized by a reduced synthetical ability and atrophy of the liver.

Bizin et al. (1977) performed a comparative study of the effect on rats of continuous and intermittent exposure to CCl_4. Continuous inhalation of 500 mg CCl_4/m^3 for 10 days induced toxicity symptoms 4- to 5-fold as rapidly as did inhalation for 6 hours daily for 40 days (Bizin et al., 1977).

Additionally, Alumot et al. (1976) reported the effects upon groups of six weanling rats (strain not given) 4 weeks old fed a diet containing CCl_4 at 150, 275 or 520 mg/kg of feed for 5 weeks (females) or 6 weeks (males). The fumigated feed was stored in airtight containers; CCl_4 loss during the storage period of 7-10 days was determined to be 5%. The animals were allowed access to the feed only at set time intervals to minimize loss of CCl_4 by volatilization. The authors calculated that the amount of CCl_4 remaining in the consumed feed was 60-70% of the amount initially present; the total decrease reflected amounts lost during storage and after removal from storage to feeding troughs. From these data and the weights of the animals, the authors calculated that 275 mg/kg of feed represented a daily dose of 40 mg/kg bw. By assuming that all parameters were the same and that the delivered dose was proportional to the concentration in feed, diets of 150 and 520 mg/kg of feed can be calculated to represent daily doses of 22 and 76 mg/kg bw, respectively (U.S. EPA, 1981). At the end of the experiment the animals were weighed and killed. Of the three doses, only the highest, 76 mg/kg bw (520 mg/kg of feed), caused significantly depressed weight gain in males. Weight gain in females appeared to be unaffected by all doses. Total lipid and triglyceride levels in the liver were significantly higher in animals fed CCl_4 at 40 and 76 mg/kg bw than in controls or animals fed 22 mg/kg bw. Levels of liver phospholipids (measured in females) were not affected at any dose. Of the three doses used in this experiment, the lowest (22 mg/kg bw) failed to produce effects on the measured parameters.

Prendergast et al. (1967) repeatedly (8 hours/day, 5 days/week) exposed 15 Hartley guinea pigs to CCl_4 at 515 mg/m³ over a period of 6 weeks and observed hepatic changes. Three guinea pigs died on days 20, 22 and 30. All the animals showed a body weight loss. The surviving animals were sacrificed at 6 weeks and the livers examined histopathologically. The examination revealed fatty infiltration, fibrosis, bile duct proliferation, hepatic cell degeneration and regeneration, focal inflammatory cell infiltration, alteration of lobular structure and early portal cirrhosis. The lipid content of the guinea pig liver was reported to be $35.4 \pm 10.7\%$, much higher than the control value of $11.0 \pm 3.6\%$.

In addition, the investigators continuously exposed 15 Hartley guinea pigs to CCl_4 vapor at 61 mg/m³ for 90 days. Three of the 15 guinea pigs died on days 47, 63 and 74. All the exposed animals showed a depressed weight gain. A "high incidence" of enlarged and discolored livers was reported on gross pathological examination. Histopathologic examination of the livers revealed fatty changes, fibroblastic proliferation, collagen deposition, hepatic cell degeneration and regeneration, and structure alteration of the liver lobule. Enzymatic studies showed that only the succinic dehydrogenase (SDH) activity was moderately reduced as compared to that in controls.

Prendergast et al. (1967) also exposed Hartley guinea pigs, number unspecified, to CCl_4 vapor continuously at 6.1 mg/m³ for 90 days. The authors reported that "no visible signs of toxicity" were seen during this study. Lipid content of the liver and serum urea nitrogen were within normal range. The authors concluded that no pathologic changes could be attributed to CCl_4 exposure.

In addition to their studies on guinea pigs, Prendergast et al. (1967) studied the effects of both repeated and continuous exposure to CCl_4 on three squirrel monkeys, three New Zealand albino rabbits, and two beagle dogs for each exposure regime. The experimental designs were the same as those described for the guinea pigs. All the animals showed a weight loss during repeated exposure to 515 mg/m³. Fatty changes were noted in the liver of all species being most severe in rabbits, followed by dogs and monkeys. In the continuous exposure to 61 mg/m³ for 90 days, all species exhibited a depressed weight gain, as did guinea pigs. Liver changes were also noted, but enzyme activities (as measured by NADH, NADPH, SDH, LDH and G6PD) were within the normal range. At a continuous exposure of 6.1 mg/m³, no toxic signs were noted.

Finally, the same investigators (Prendergast et al., 1967) studied the effects of repeated (515 mg/m³) and continuous (61 mg/m³ or 6.1 mg/m³) exposure on 15 Long-Evans or Sprague-Dawley rats following the same methodology described above. With repeated exposure, there was a high percentage of mottled livers. Histopathologic examination revealed morphologic changes in lungs and livers but no changes in the heart, spleen or kidney. Fatty changes also developed in the liver. Following continuous exposure to 61 mg/m³, depressed growth curves resulted as compared to control animals.

Examination upon autopsy revealed a high incidence of enlarged and/or discolored livers. Histopathologic liver changes included fatty infiltration, fibroblastic proliferation, collagen deposition, hepatic cell degeneration and regeneration, and alteration in the structure of the liver lobule. There were no adverse effects following continuous exposure to 6.1 mg/m³.

The studies done by Prendergast et al. (1967) have been criticized due to small sample size (only rats and guinea pigs had numbers >10), inconsistent reporting (over 10 different descriptions of liver damage were mentioned) and vague information such that only the general conclusion that liver damage follows CCl_4 inhalation can be made (EnviroControl, 1981).

8.1.3. Chronic. Smyth et al. (1936) studied the toxicity of CCl_4 on rats after chronic inhalation exposure. Groups of 24 Wistar rats were exposed to CCl_4 concentrations of 315, 630, 1260 or 2520 mg/m³ for 8 hours/day, 5 days/week for 10.5 months. The CCl_4 was found to contain <0.003% carbon disulfide. Control rats were used, but the number was unspecified. Growth retardation was observed with the 2520 mg/m³ dose. At the 630 and 1260 mg/m³ dose, growth was the same as in controls, and at the 315 mg/m³ dose the growth was stimulated. Cirrhosis developed in the 630, 1260 and 2520 mg/m³ groups after 173, 115 and 54 exposures, respectively, but not in the 315 mg/m³ group. When exposure was stopped fatty liver degeneration resolved within 50 days. Surface alterations (hobnail liver) did not resolve until 156 days after cessation of exposure. Unspecified renal damage was observed after 52 exposures to the 315 mg/m³ concentration and after 18-20 exposures at the higher concentrations, but was termed "not extreme."

In this same study, groups of 24 guinea pigs (strain not given) were exposed to CCl_4 vapor at 315, 635, 1260 or 2520 mg/m³. The frequency of exposure was 8 hours/day, 5 days/week for ≤10.5 months. Marked mortality occurred in exposed animals: 9/24 at the 315 mg/m³ dose after a median of 44 exposures (exposure terminated at 135 days), 16/24 at the 630 mg/m³ dose after a median of 10 exposures, 13/24 at the 1260 mg/m³ dose after a median of three exposures, and 19/24 at the 2520 mg/m³ dose also after a median of three exposures.

The guinea pigs exposed to the 315 mg/m³ dose developed cirrhosis and hobnail surface alterations of the liver in 105 exposures. The authors concluded that survival of guinea pigs at higher doses was of insufficient duration to allow development of cirrhosis. In addition, granular swelling was observed in adrenal glands of guinea pigs exposed to CCl_4 at 315, 630 and 1260 mg/m³ for 8, 7 and 17 exposures, respectively. Exposure to higher concentrations (1260 or 2520 mg/m³) or continued exposure to lower concentrations resulted in marked damage to the sciatic nerves. Dense clumps of black granules (osmic acid stain) were observed paralleling the large majority of fibers.

Rhesus monkeys were also examined. They inhaled CCl_4 at concentrations of 320 and 1280 mg/m³ for 8 hours/day, 5 days/week for 10.5 months (Smyth et al., 1936). Monkeys exposed to 1280 mg/m³ showed an 8% less weight gain than the controls. At both 320 and 1280 mg/m³, monkeys had livers with slight fatty degeneration following 8.7 months of exposure. The livers returned to normal 28 days postexposure. Two of four monkeys exposed to 1280 mg/m³ for 8.7 and 10.5 months showed definite damage to the sciatic nerve.

In summary, guinea pigs were found to be more sensitive to the toxic effects of CCl_4 than were rats or monkeys. This may be due, in part, to the diet provided to the guinea pigs (low in calcium and lacking in animal fat and protein). All concentrations provided, produced some effect in the rats, and very little harm was observed in the monkeys. The liver and kidney injury produced in the monkeys reversed completely 28 days following exposure.

In a chronic oral exposure study (Alumot et al., 1976), groups of 36 rats (18 male and 18 female littermates, strain not given) were fed mash containing CCl_4 at 0, 80 or 200 mg/kg of feed. The feed was stored in airtight containers, assayed for CCl_4 content, and consumed soon after removal to feeding troughs. The authors calculated that the 200 mg/kg of feed represented a daily dose of 10-18 mg/kg bw. During this 2-year study, several parameters were measured. No effects were noticed on body weight gain for up to 13 weeks. Throughout the study measurements of male and female fecundity remained essentially normal. After 2 years, the surviving animals were killed. In these animals, serum values for glucose, protein, albumin, urea, uric acid, cholesterol, SGOT and SGPT in the treated animals did not differ from those in controls. No fatty livers were detected in the treated animals. Thus, the authors found no biochemical, histopathologic, reproductive or other abnormalities attributable to CCl_4 exposure. However, interpretation of the results was complicated by the widespread incidence of chronic respiratory disease in the animals which started at about 14 months into the experiment. More than half the animals were dead at 21 months, although at 18 months the survival ranged from 61-89%. Although the authors indicated that 10-18 mg/kg bw (200 mg/kg of feed) is a no-observed-adverse-effect level (NOAEL) of CCl_4 over 2 years, this conclusion may be questioned because of the chronic respiratory infection and hence poor survival of the animals in the latter part of the experiment.

Yet, it may be inferred from these results that a level of 10-18 mg/kg bw/day over a 1-year period caused no observable adverse effects.

A chronic inhalation study by Adams et al. (1952) also presented incomplete data. No numbers of animals tested, surviving, or affected were given; therefore, it is not possible to determine what measurements were made at different exposures. The only conclusion possible is that at some exposures ranging from 32.5-2600 mg/m³, 7 hours/day, 5 days/week for 258 days, some liver damage occurred in albino Wistar rats, albino rabbits (strain not given) and guinea pigs (strain not given). A few rhesus monkeys were tested, but only limited information is presented. At least one monkey exposed to 25 ppm CCl_4, 7 hours/day, 5 days/week, survived for 212 days with no apparent adverse effects. The limited information and confusing description precludes a more detailed appraisal of the study.

In a study previously discussed (Merkur'eva et al., 1979), the authors also examined the effect of chronic CCl_4 inhalation. Long-term exposure to 300 mg CCl_4/m³ caused a considerable increase in the DNA-synthesizing connective tissue cells (Merkur'eva et al., 1979).

Rotenberg (1978) examined the dynamics of liver bioenergetic system responses following the chronic exposure of 150 rats (strain not given) to small concentrations of CCl_4 in air (14 mg CCl_4/m³, 5 hours/day, 5 days/week for 5 months). As stated by the authors, the exposure caused phasic changes in hepatic energy-producing processes as evidenced by alterations in respiratory rate, phosphorylation and sensitivity of respiratory enzymes to respiration inhibitors.

8.2. HUMANS

Many poisonings have resulted from the accidental or suicidal ingestion of CCl_4 or from its medicinal use as an anthelmintic. For its medicinal use, the therapeutic dose recommended for adults was 2-3 mℓ in capsule form and 0.13 mℓ/year for infants and children up to 15 years of age (von Oettingen, 1964). As emphasized by von Oettingen (1964), such doses, which are followed by doses of Epsom salts, have caused toxic effects only exceptionally. Horrocks (1934) reported one fatality from its medicinal use. The vast majority of poisonings, however, have resulted from the inhalation of its vapors when used as a solvent or dry cleaning agent (von Oettingen, 1964). Still other poisonings have been the result of dermal exposures through the use of CCl_4 in shampoos (NIOSH, 1975). Finally, some have resulted from its use in fire extinguishers (Dudley, 1935).

Norwood et al. (1950) reported the occurrence of 2 fatalities, 1 near fatality, 4 poisonings requiring hospitalization, and 51 mild industrial poisonings in two communities over a period of 1 year. In 1935, Smyth (1935) noted 28 fatalities, 14 of which resulted from the ingestion of CCl_4; 120 acute and subchronic poisonings; and 7 cases of chronic poisoning. By 1964, an additional 28 poisonings resulting from CCl_4 ingestion (including 10 fatalities) and 202 cases from inhalation (including 29 fatalities) were reported. The actual incidence of such poisonings was doubtless much greater, since many poisonings were not attributed to CCl_4 and others were not published in the medical literature (von Oettingen, 1964). Since 1964, there have been additional poisonings and case reports (Bagnasco et al., 1978; Bonitenko and Bruk, 1979; Shimanko et al., 1979; Campbell et al., 1980); however, the total number has not been compiled.

8.2.1. Case Reports.

8.2.1.1. **ACUTE** -- Oral poisonings from acute exposure to CCl_4 have occurred to a great extent, as reported by a number of authors (Docherty and

Burgess, 1922; Beattie, et al. 1944; NIOSH, 1975; Kirkpatrick and Sutherland, 1956; Dawborn et al., 1961). A summary of the symptoms of such oral poisoning is given below (von Oettingen, 1964). Following ingestion of CCl_4, the patient experiences a burning sensation in the mouth, esophagus, and stomach. Depending upon the dose, this is sooner or later complicated by abdominal pain, nausea, and vomiting. Some patients develop hiccoughs. The tongue is coated. These symptoms are soon followed by diarrhea, which later may be followed by constipation and occasionally by gastric and intestinal hemorrhages which, in rare cases, may also be seen in the mouth and pharynx. Again, depending upon the dose along with other factors, the patient becomes jaundiced, the liver becomes enlarged and tender; this may be associated with ascites and generalized edema. Soon after the ingestion, the patient feels dizzy, may suffer from headache and become confused, semiconscious and delirious. The patient may become restless and develop choreatic movements. Finally, consciousness is lost and the patient passes into coma. Some patients complain of visual disturbances and edema of the eyelids and develop hemorrhages of the sclerae. In severe cases, circulatory disturbances may develop, characterized by lowered or increased blood pressure, thin and rapid pulse, and signs of congestive heart failure with cyanosis.

A fatality attributed to ingestion of CCl_4 was reported by Smetana (1939). The victim, a photographer described as having "a history of chronic alcoholism," died 10 days after consuming an unknown amount of "some fluid containing carbon tetrachloride." He presented symptoms including nausea, vomiting, jaundice, anuria and semistupor. In the final clinical diagnosis, death was attributed to CCl_4 poisoning.

A case of attempted suicide by ingestion of CCl_4 was reported by Stewart et al. (1963). The victim, a 29-year-old female who ingested one pint of a CCl_4:methanol solution (2:1), experienced ringing in the ears immediately after ingestion and lost consciousness. She was hospitalized for 3 weeks. Three hours after ingestion, CCl_4 in the exhaled breath and blood was confirmed by infrared analysis. The exhaled breath was then monitored throughout the hospitalization; CCl_4 was reported to decrease exponentially. Because of the toxicity of the methanol and the possibility of synergistic reactions with the CCl_4, hemodialysis was performed soon after admission. Mannitol solution was given by continuous intravenous infusion. Clinical laboratory analyses during hospitalization showed some elevation of SGOT, which reached a maximum of 75 units on day 6, and an elevation of urinary urobilinogen to a maximum of 7.8 Ehrlich units on day 10. Other laboratory findings included elevation of serum iron and depression of serum protein concentration and albumin fractions. The retention time of bromosulfophthalein was increased. These findings were interpreted as evidence of minimal hepatocellular injury. Acute renal dysfunction was not observed; the authors credited the mannitol treatment with preventing renal damage.

Lamson and Minot (1928) studied the lethal effects of CCl_4 on patients receiving CCl_4 and magnesium sulfate orally as a treatment for hookworms. The authors reported the treatment of thousands of patients with a single dose of 2.5-15 mℓ CCl_4 without ill effects. One man was reported to have safely ingested 40 mℓ of CCl_4. However, an "extremely small" population of adults died after receiving 1.5 mℓ of CCl_4; doses of 0.18-0.92 mℓ were reported to be fatal to children. Susceptibility in adults was correlated with alcoholic intake (chronic alcoholism or exposure to alcohol shortly after treatment), the presence of ascarid worms, and the intake of foods, particularly of high fatty content.

There are few postmortem reports on pathological changes in patients after the ingestion of CCl_4. McMahon and Weiss (1929) examined a 34-year-old male alcoholic who died 5 days after drinking one ounce of CCl_4. They discovered some reddish-brown fluid in the abdominal cavity, early atheromatous lesions in the heart, congested and edematous lungs with scattered petechial hemorrhages, enlarged and congested kidneys, marked erosion of the esophagus, and a congested and enlarged fatty liver.

Cases of acute toxicity associated with CCl_4 inhalation by humans have been more numerous. Bilateral peripheral constriction of the ocular color fields, resulting in symptoms of toxic amblyopia in three males, was attributed to the inhalation of CCl_4 vapors (Wirtschafter, 1933). Five male employees of dry cleaning establishments who had been exposed to CCl_4 (of unknown concentration) from 8-10 hours daily for 1-6 months were examined. Two men also had signs of conjunctivitis. Three of the men complained of visual disturbances characterized by blurred vision or spots before the eyes. Wirtschafter concluded that toxic amblyopia may result from exposure to CCl_4 vapor.

One fatality occurred in two cases of CCl_4 poisoning reported by Smetana (1939). In the fatality, a dry cleaner and interior decorator described as being "a steady and heavy drinker" was exposed for several hours to CCl_4 vapors (concentration unknown) during work. Upon returning from work, he noted dyspnea. Several hours after the exposure, headache, dizziness and malaise developed, accompanied by nausea and repeated vomiting that persisted for several days. The patient also suffered labored breathing and cough with bloody sputum before he died 9 days after exposure.

The second inhalation case reported by Smetana was a housemaid also described as having a history of chronic alcoholism. Three days before hospitalization, the patient cleaned dresses with CCl_4 for 3 hours in a poorly ventilated room. Soon after exposure, she began to vomit. She suffered symptoms similar to those described for the other case. After ~1.5 months of hospitalization, this patient was released; her condition several weeks later was described as "much improved."

Seven of the cases of CCl_4 poisoning reported by Norwood et al. (1950) resulted from both occupational and nonoccupational inhalation exposures. In the three cases described as "severe" poisonings, there was a history of chronic alcoholism; two fatalities occurred in this group. In one fatal case, the victim had been exposed for about 25 minutes to an atmosphere containing CCl_4 at an estimated 1575 mg/m^3 (this estimate was made by duplicating the conditions). Histopathological examination of liver and kidney tissue from the fatalities revealed liver necrosis and degeneration of the renal tubules. The four remaining cases were characterized as "mild industrial" exposures. After CCl_4 exposure, all subjects suffered varied symptoms including nausea, vomiting, diarrhea, headache, muscular ache, pain or numbness, labored breathing and dizziness.

In another case, a 31-year-old janitor suffered malaise, back and lower abdominal pain, nausea and vomiting the morning after working for 5 hours in a closed room with an unknown concentration of CCl_4 (Kittleson and Borden, 1956). He reportedly consumed two bottles of beer during the exposure period. The patient required 2 months of hospitalization for treatment of acute renal insufficiency as a result of CCl_4 intoxication.

Elevated SGOT activities with concomitant liver changes were reported in two men occupationally exposed to unreported concentrations of CCl_4

(Lachnit and Pietschmann, 1960). One became ill after exposure to CCl_4 for 3 hours in a relatively well-ventilated room. He was hospitalized 3 days after exposure. His liver was slightly enlarged, with the SGOT value elevated by 6000 units. This value rapidly decreased and by the 10th day had returned to normal. A biopsy of the liver taken on the 8th day showed necrosis in the centers of the lobuli, but the surrounding tissue was undamaged. An additional needle biopsy of the liver taken on the 28th day showed that the cells had almost returned to normal. In the second case a male similarly exposed to CCl_4 entered the hospital 12 days after exposure. The SGOT had increased to 80 units. A liver needle biopsy on the 22nd day showed only moderate changes, some of a degenerative nature.

In a chemical packing plant, use of CCl_4 by two workers for equipment cleaning as a substitute for the customarily used acetone, resulted in the hospitalization of 4 of 43 workers at the plant (Folland et al., 1976). Ten additional workers became ill. Eight of the 43 workers fell ill within 12 hours following the start of the 2-hour exposure; six others followed within the next 36 hours. The four hospitalized workers showed evidence of severe disruption of liver function: one case had an SGOT level of 13,390 units. All patients recovered within 90 days. All hospitalized workers, as well as most of the others taken ill, had worked near a bottle-filling operation for isopropyl alcohol at the northern end of the plant, adjacent to the CCl_4 cleaning area.

Carbon tetrachloride concentrations at the time of exposure were not ascertained; acetone was normally used for cleaning. Isopropyl alcohol concentrations at the northern end of the plant averaged 2624 mg/m^3. Acetone in alveolar air samples of workers in the northern area averaged 121.6 mg/m^3. The authors ascribed the toxic episode to CCl_4 toxicity potentiated by isopropyl alcohol. Because CCl_4 concentrations were unknown and isopropyl alcohol (and possibly other chemicals) were present, the health effects reported in this study cannot be attributed to CCl_4 exposure alone.

Some of these reports and others (Davis, 1934; Stewart et al., 1961; Smith, 1950; NIOSH, 1975; von Oettingen, 1964) indicate that with single exposure to low concentrations, there is considerable variation in symptoms among different persons and that the acute toxicity is relatively low in contrast to that with repeated exposure. Cases in which exposure is light may be restricted to such symptoms as moderate irritation of the eyes, moderate dizziness and headache, which disappear promptly upon discontinuation of the exposure.

The immediate effects from acute inhalation exposure to higher concentrations of CCl_4 consist of the same symptoms as described above; however, the patient may also become nauseated and suffer from loss of appetite, mental confusion, agitation and the feeling of suffocation. In severe cases, the patient may lose consciousness and develop fever and chills. The tongue may be furred and the patient may eject bloody or bile-stained vomitus which may last for days, and experience colicky pain and diarrhea with liquid brown-black or bloody stools (von Oettingen, 1964). This tendency for hemorrhages may also result in bleeding from the gums and nose, hemorrhages under the skin and macular papular rashes. The colicky pain may be associated with a marked abdominal resistance simulating the "acute abdomen" and thus has been mistaken for appendicitis and peptic ulcer. Following such an acute episode, the patient feels tired and weak and frequently suffers from headache. The patient may develop muscular twitchings and epileptic convulsions. In a few instances, paralysis (hemiplegia) and polyneuritis have been reported (von Oettingen, 1964).

In more severe inhalation poisoning blood pressure may be lowered, but as renal complications develop, the blood pressure is usually elevated and the cardiac output is decreased because of increased peripheral resistance. The pulse may be accelerated. In the case of severe inhalation poisoning, the patient may collapse. Electrocardiograms have shown changes characteristic of myocardial injury characterized by sinus bradycardia and followed by auriculoventricular arrhythmia, auricular fibrillation and sinus arrhythmias (von Oettingen, 1964).

Depending upon the condition of the patient, respiration may be normal, rapid and shallow, or slow and labored. The latter is evident especially if circulatory failure is imminent and pulmonary edema develops. Thompson (1946) found that early roentgenograms of the lungs may show pulmonary involvement.

In most instances after severe inhalation exposure, the patient develops signs of liver injury within a few days. The patient becomes jaundiced and the liver becomes enlarged and tender. This is toxic hepatitis, which may pass into yellow atrophy and, in more protracted cases, eventually into cirrhosis of the liver. In the early stages of liver injury, even before a marked enlargement occurs and while liver function tests such as the cephalin-flocculation test are still normal, the SGOT level may be markedly elevated (von Oettingen, 1964).

As signs of liver injury develop, and sometimes in their absence, injury of the kidneys may dominate the clinical picture and be responsible for early death (von Oettingen, 1964). Kittleson and Borden (1956) characterized renal failure by three phases. The first phase is characterized by polyuria and nocturia, which may result in severe dehydration, followed by oliguria and finally by diuresis. The renal injury may result in acute nephritis with albumin, red and white cells, and casts in the urine. In some patients, the presence of acetone and sugar in the urine has been reported. The oliguria may be associated with increased blood levels of potassium, indican, phenol, cresol, creatinine and urea; the latter may result in uremia. In other instances, the injury may consist of necrotizing nephrosis with comparatively little change in the urinary composition. The renal blood flow and glomerular filtration rate are decreased; and the former seems to be mainly responsible for the maintenance of oliguria, being the sequela rather than the cause of renal failure (von Oettingen, 1964). During the early stage of oliguria, abnormal tubular back diffusion of the filtrate may play an important role. Oliguria may develop as early as 24 hours or 3-4 days after onset of the poisoning and may persist for 12-14 days and even longer (von Oettingen, 1964).

In the early stages after severe inhalation poisoning and during the period of polyuria, the blood may show some polycythemia, but later this may be followed by anemia and lowering of the hematocrit levels because of hemodilution. However, the most important changes in the blood are related to the biochemical composition of the blood which reflects the renal and hepatic injury. As soon as the renal injury develops, the nonprotein nitrogen and urea nitrogen levels in the blood are increased and may reach extremely high values. The creatinine, indican, phenol and cresol levels may also be increased. In the case of liver injury, as related to the blood, the icteric index is usually increased, and the levels of sugar and phospolipids, along with the ratio of cholesterol esters over cholestrol, are reduced. The prothrombin time and the fibrinogen content may be reduced, resulting in an increased clotting time. The chloride level is frequently lowered by hemodilution or severe vomiting, and the potassium level may be elevated. This increase in potassium may contribute to ventricular fibrillation or cardiac arrest (von Oettingen, 1964).

In addition to constriction of the visual field and toxic amblyopia (Wirtschafter, 1933), CCl_4 poisoning can also result in blurred and double vision. Conjunctival hemorrhages are common. Retinal hemorrhages and exceptional cases of the degeneration of the optic nerve have been reported (von Oettingen, 1964).

Conway and Hoven (1946) report that after ingestion of CCl_4, certain electrocardiographic changes may be observed indicating degenerative processes in the heart muscle, such as sinus bradycardia, followed by auriculoventricular rhythm, auricular fibrillation, and sinus arrhythmias. The respiration varies with the condition of the patient. If the patient is in collapse, it will be rapid and shallow; if the patient is comatose, it may be labored and dyspneic, and pulmonary edema and hemorrhages may develop. Eventually, disturbances develop characterized by polyuria and followed by oliguria which may pass into anuria. The urine of such patients is rich in albumin and may contain blood and casts. If the liver is damaged, the urine will contain urobilinogen, urobilin, and bile pigments. The nonprotein nitrogen level in the blood will be increased and the patient may suffer from hypoprothrombinemia, hypochloremia, and signs of acidosis. Death may ensue after 8 hours, or 3, 5 or 10 days, and sometimes later.

Two cases involving the pancreas following inhalation of an acute exposure of CCl_4 were reported by Jahnke (1953). Both patients became listless and developed hepatic and circulatory disturbances and sensitivity of the pancreas to pressure. Such disturbances were long-term and had not completely subsided after 10 months.

Guild et al. (1958) reported on 20 cases of CCl_4 intoxication which resulted in renal damage manifested by anuria. Four of these were the result of ingestion. Exposures varied: one drank 15 cc; one drank 4 ounces; two drank small amounts but were also consuming alcohol (see Chapter 12). The remaining 16 cases inhaled CCl_4 with exposure times ranging from 30 minutes to 11 hours. Two of the four that ingested the CCl_4 died whereas three died of the 16 exposed through inhalation.

Severe liver involvement was seen in all four of those ingesting CCl_4. Eleven of the 16 who inhaled CCl_4 had "moderate to severe" liver involvement whereas the remaining 5 had no liver involvement (Guild et al., 1958). These discrepancies can be attributed in part to the variability in exposure amounts and duration; however, this cannot be resolved from the report. The role of alcohol consumption also cannot be defined, although this report does lend some support to the research discussed in Chapter 12.

Stevens and Forster (1953) reported on the neurological effects of CCl_4. Fifteen cases of CCl_4 intoxication with varying amounts and routes of exposure are discussed. Thirteen occurred in the home and two occurred in commercial cleaning plants. Of the 13 cases occurring at home, 2 were children (the youngest being 9 months old) who drank the fluid. Two were adults who accidentally drank it, and the others were exposed via inhalation. There were five deaths. Eleven of the 13 were alchoholic. Four of the deaths occurred in alcoholics; for the fifth, no history was available. Seven of the 15 experienced neurological symptoms. Of these, two children who ingested the chemical displayed stupor, drowsiness or unconsciousness. The adults displayed headache, vertigo, weakness, blurred vision, lethargy and coma.

8.2.1.2. SUBCHRONIC/CHRONIC -- Subchronic/chronic human exposures to CCl_4 are nearly always by inhalation. Headache, fatigue, dizziness, nausea, and vomiting occur at substantially lower concentrations in these exposures than in acute exposure (Elkins, 1942; Kazantzis and Bomford, 1960). As in acute exposures fatty and necrotic livers are the common pathological findings (Gray, 1947; Dellian and Wittgens, 1962; Barnes and Jones, 1967). In some cases, renal injury is the major finding. Renal tubular necrosis has been reported both with and without concomitant liver disease (Hamburger et al., 1958; Richet et al., 1959; von Oettingen, 1964).

Carbon tetrachloride poisoning as a result of chronic inhalation of low dose exposures has been reported by Butsch (1932), Wirtschafter (1933), Straus (1954), von Oettingen (1964) and others. The clinical picture after chronic CCl_4 exposure is much less characteristic than that after acute exposure. With chronic exposure, patients may complain of fatigue, lassitude, giddiness, anxiety, and headache. They suffer from paresthesias and muscular twitchings and show increased reflex excitability. They may be moderately jaundiced, have a tendency to hypoglycemia, and biopsy specimens of the liver may show fatty infiltration. Patients may complain of lack of appetite, nausea, and occasionally diarrhea. In some instances, the blood pressure is lowered accompanied by pain in the cardiac region and mild anemia. Other patients develop pain in the kidney region, dysuria and slight nocturia and have urine containing small amounts of albumin and a few red blood cells. Burning of the eyes and, in a few instances, blurred vision are frequent complaints of those exposed. If these symptoms are not pronounced or of long standing, recovery usually takes place upon discontinuation of the exposure if the proper treatment is received (von Oettingen, 1964).

Straus (1954) suggested a possible causal relationship between CCl_4 exposure and aplastic anemia. Three males had been exposed via inhalation and dermal absorption to CCl_4 at unknown concentrations for 2 months to 3 years. Autopsy findings included hypoplasia of the bone marrow. However, a causal relationship between CCl_4 and aplastic anemia suspected by the author in these cases is not supported adequately. One of the men had also been exposed to kerosene for 3 years. Another was an auto mechanic who worked in a garage. The occupation of the third was not specified although his exposure to CCl_4 was occupationally-related. Thus the effects of other chemicals cannot be discounted. The autopsy findings of two of the patients included no liver or kidney damage of the type that would be expected in CCl_4 poisoning. In the third case the liver was reported to have periportal fibrosis and fatty infiltration. These findings were attributed to toxic hepatitis which was considered to be the result of CCl_4 poisoning. The information reported in these case studies tends not to substantiate the author's suggestion that the patients' illnesses may have been caused by CCl_4.

Postmortem reports on pathological changes in patients after inhalation of CCl_4 are generally limited to findings in the liver and kidneys. The liver may show nutmeg appearance and fatty degeneration even in the absence of clinical signs and symptoms of liver injury. In other instances, centrilobular necrosis and hemorrhages with infiltration of leukocytes and histiocytes and collapse of the lobules with condensation of the reticular framework within these areas are seen. After chronic exposure, there may be evidence of regeneration of the liver cells (von Oettingen, 1964).

Postmortem changes in the kidney are characterized by nephrosis, by a distention of Bowman's capsule with albuminous precipitates, and by swelling of the lining cells. The cells of the convoluted tubules may be swollen and vacuolated; later, degenerative changes may be seen in Henle's loops, associated with granular, hyaline, and cellular casts in the tubules. After chronic exposure, regenerative changes may be visible in these regions. In other cases, the kidneys may offer the picture of acute hemorrhagic nephritis (von Oettingen, 1964).

Other postmortem organ changes are less characteristic for CCl_4 poisoning and vary considerably with the clinical picture. Some changes may occur that are a direct result of the changes occurring in the primary target organs of CCl_4. Stasis of various organs is the most outstanding feature of cardiac failure. The brain and lungs may be edematous. The intestines may be hyperemic and covered with numerous petechial hemorrhages, and the spleen may be enlarged and hyperemic. Occasionally the adrenal glands may show degenerative changes of the cortex, and the heart may undergo toxic myocarditis (von Oettingen, 1964).

8.2.2. Controlled/Clinical Studies. Human volunteers were exposed to known concentrations of CCl_4 vapor in an effort to correlate physiological and/or biochemical changes to the magnitude of exposure (Stewart et al., 1961). Eight healthy male volunteers were exposed to CCl_4 vapors in a series of three separate experiments performed 1 month apart. Prior to exposure, data on blood pressure, SGOT and urinary urobilinogen were obtained for each subject. Prior to exposure, samples of exhaled breath, urine and blood showed no detectable CCl_4. The volunteers were seated in a closed room (11 x 12 x 7.5 feet) where 99% pure CCl_4 was poured into a dish and covered with a towel. An exhaust system grill and door were closed during the experiment but an air supply grill was left open. A fan circulated air across the dish. Ambient concentrations of CCl_4 were monitored with a Davis halide meter and an infrared spectrometer. The CCl_4 concentration ranges and exposure times are given in Table 8-6.

Carbon tetrachloride was detected in exhaled breath in all three experiments. Graphs showed an exponential decrease in concentration of CCl_4 versus time. The exact values were not given.

The serum iron showed an initial decrease in 3 of 6 subjects at the 309 mg/m³ exposure level but had returned to normal in two of these subjects 68 hours after exposure. The remaining subject showed a 31% depression in serum iron at 68 hours, but the value was within the normal range. Serum iron was not analyzed in the other two experiments. Of the six subjects exposed to 309 mg/m³ CCl_4, the serum transaminase level was slightly elevated in some and depressed in others, but remained within the normal range. Carbon tetrachloride was not detected in the blood or urine at any exposure time or dose, but the analytical technique used (infrared method) was not a sensitive one. The authors concluded that no ill effects were observed from exposure to CCl_4 at 63 mg/m³ for 180 minutes, although the small changes in serum iron at the 309 mg/m³ dose might have been an indication of liver insult.

A survey conducted by Kazantis and Bomford (1960) was the result of the complaints of a worker in a factory processing raw quartz. Intermittently for 2 years, he experienced anorexia, nausea and occasional vomiting with abdominal discomfort. He noticed that his symptoms or dyspepsia worsened during the workweek but got better on the weekends. The investigators interviewed 17 of the 18 employees working in the same area taking medical and occupational histories; their ages ranged from 16-54. Fifteen of the 17 complained of symptoms similar to the initial case, primarily nausea, anorexia, vomiting, flatulence, epigastric discomfort and depression. These

TABLE 8-6

Exposure Times and Concentrations of Carbon Tetrachloride Vapor In a Controlled Human Study*

Experiment	Average Concentration, Time-weighted (mg/m³)	Concentration Range (mg/m³)	Exposure (minutes)
1	309	192-548	70
2	69	63-88	180
3	63	57-88	180

*Source: Stewart et al., 1961

symptoms had been occurring among the workers from 1 week to 2 years. Environmental monitoring measured CCl_4 atmospheric concentrations of 292.5-650 mg/m³. Once the cause of the high concentration was found, changes were made in the processing procedure. Within 1 week following these changes, the symptoms disappeared in all workers. Follow-up for 6 months revealed no recurrences.

Direct application of CCl_4 to human skin causes a burning and stinging sensation within 5 minutes. The maximum pain is reached 6 minutes later and is associated with erythema, hyperemia, and wheal formation, later followed by vesication (Oettel, 1936).

The absorption of CCl_4 through human skin was measured by immersion of the thumbs of three male and female volunteers in a sample of this compound for 30 minutes (Stewart and Dodd, 1964). The CCl_4 was analyzed by infrared spectroscopy, and no impurities were detected. Sequential sensations of burning and cooling were experienced by all volunteers during the immersion. Burning ceased about 10 minutes after removal from the solvent. The thumbs of all volunteers appeared scaly and red, a condition that improved within several hours after exposure. Carbon tetrachloride was detected in the alveolar air of each subject within 10 minutes of immersion of their thumbs. The concentration in the expired breath rose continuously to a maximum of 4.0 mg/m³ 10-30 minutes after the exposure period ended, and then decreased exponentially. The mean concentration of CCl_4 2 hours after the end of exposure was 2.0 mg/m³; at 5 hours after exposure, the alveolar air concentration was still >0.6 mg/m³. The authors concluded that CCl_4 could be absorbed through the skin in toxic quantities.

Hall (1921a,b) demonstrated the effectiveness of CCl_4 as a vermicidal agent in treatment of hookworm infestations. The usage of CCl_4 in this capacity stimulated considerable research efforts to investigate the pharmacologic and physiologic effects of CCl_4 on humans (NIOSH, 1975). The effects of oral doses of CCl_4 as a human anthelmintic, administered to condemned prisoners in Ceylon has been reported (Docherty and Burgess, 1922; Docherty and Nicholls, 1923). Three of the prisoners received 4 mℓ CCl_4, two received 5 mℓ CCl_4, and one received 5 mℓ plus an additional 3 mℓ 2 weeks after the first dose. Execution of the prisoners occurred 3-15 days after the CCl_4 administration. Autopsies were performed and the findings varied. The livers of some showed no major microscopic or macroscopic changes whereas the livers of others showed marked fatty degeneration. From such data, a dose-response relationship would be difficult to determine (NIOSH, 1975).

A cross-sectional epidemiologic study (Sonich et al., 1981) examined the effect upon persons exposed to CCl_4 through their drinking water. Seventy tons of CCl_4 were spilled in the Kanawha and Ohio Rivers in 1977. Measurements of raw water revealed maximum concentrations of 0.340 mg/ℓ. Twenty-one cities situated along the river were involved in the study. These cities represent areas that draw their drinking water directly from the river and/or areas that draw their drinking water from sources not influenced by the quality of the river water. Measurements at each of the four major cities along the river showed a decrease in the CCl_4 concentration in the river with the number of river miles from the spill: Huntington, West Virginia - 0.210 mg/ℓ; Cincinnati, Ohio - 0.180 mg/ℓ; Louisville, Kentucky - 0.110 mg/ℓ; Evansville, Indiana - 0.060 mg/ℓ. Finished drinking water in Cincinnati contained a peak level of 0.087 mg/ℓ. A control period was identified in 1976 when there were nondetectable to trace amounts of CCl_4 in the river as indicated by monitoring efforts at Cincinnati. By using river volumes and flow rates, periods of high exposure (1977) and low exposure (1976) to CCl_4 were estimated for each city along the river. A total of 35 hospitals in these cities provided medical data on patients admitted during these estimated time periods. The results of routine tests measuring serum chemistries reflecting liver and kidney function along with basic epidemiologic information were abstracted from ~6000 medical records. It is hypothesized that CCl_4 would cause a rise in some or all of these serum chemistries. The data were categorically analyzed to test for a dose/response relationship. In this capacity, the ratio of the odds ratios (ROR) for each serum chemistry for each city group were computed as ROR = OR_{77}/OR_{76} where

$$\text{odds ratio}_{76} = (OR_{76}) = \frac{\text{the odds of having an elevated test result while drinking Ohio River water in 1976}}{\text{the odds of having an elevated test result while drinking water in 1976 not affected by the quality of the Ohio River}}$$

and

$$\text{odds ratio}_{77} = (OR_{77}) = \frac{\text{the odds of having an elevated test result while drinking Ohio River water in 1977}}{\text{the odds of having an elevated test result while drinking water in 1977 not affected by the quality of the Ohio River.}}$$

The results obtained for creatinine show a positive [illegible] statistically significant ($p<0.05$) dose/response relationship between the CCl_4 exposure and the ROR or frequency of elevated levels of serum creatinine in exposed patients in relation to the controls as determined by a test for linearity of trend. Other parameters analyzed were alkaline phosphatase, total bilirubin, blood urea nitrogen, lactic dehydrogenase, SGOT and γ-glutamyl transpeptidase. No similar results were found for these parameters.

8.3. MECHANISMS OF TOXICITY

The toxicity of CCl_4 to an organism depends upon the ability of the organism to metabolize the compound; unmetabolized CCl_4 does not appear to be significantly toxic (Recknagel and Glende, 1973). In mammals, as discussed in Chapter 7, CCl_4 is thought to be metabolized in the endoplasmic reticulum of the liver by the mixed-function oxidase system of enzymes. A reaction sequence proposed in the literature for CCl_4 metabolism is outlined in Figure 7-1. Two free radicals have been postulated as metabolic intermediates: the trichloromethyl radical and the chlorine radical. The toxicity of CCl_4 has been attributed to subsequent reactions of the trichloromethyl radical. These reactions include formation of carbonyl chloride (phosgene), dimerization to hexachloroethane, free radical binding to protein, and lipid peroxidation. In this chapter each of these proposed pathways will be presented in conjunction with the toxic effects attributed to it.

8.3.1. **Formation of Carbonyl Chloride (Phosgene).** From the results of an _in vitro_ study of CCl_4 metabolism, Shah et al. (1979) postulated the formation of carbonyl chloride from the trichloromethyl radical. As previously discussed, the authors incubated L-cysteine and $[^{14}C]CCl_4$ with rat liver homogenate and looked for the formation of 2-oxothiozolidine-4-carboxylic acid. This compound is formed from the reaction of L-cysteine and carbonyl chloride. Analysis of the metabolic products by mass spectroscopy showed a fragmentation pattern consistent with 2-oxothiozolidine-4-carboxylic acid. The authors inferred from these analytical data that carbonyl chloride was formed as a metabolic product of CCl_4. Although carbonyl chloride (phosgene) is not reported to be a carcinogen, the authors pointed out that the compound is highly toxic and that the reactive chlorines could react with macromolecules in ways similar to alkylating agents.

8.3.2. Dimerization to Hexachloroethane. Hexachloroethane has been identified as a metabolite of CCl_4 by Fowler (1969). The formation of this compound is believed to take place by the dimerization of the trichloromethyl radical. Although hexachloroethane is a hepatotoxin, its toxicity is less than that seen in CCl_4 poisoning. Therefore, other mechanisms probably account for the severe toxicity of CCl_4.

8.3.3. Free Radical Binding to Proteins. Free radical binding to proteins has been postulated as one cause of toxicity associated with CCl_4 (Recknagel and Glende, 1973). The binding was reported to involve reactions with cellular proteins, particularly those with sulfhydryl groups. Delvillarruel et al. (1977) found a good qualitative correlation between intensity of CCl_4 activation and P-450 content of various organs in male, virgin female, or pregnant female rats. No correlation was found between CCl_4 activation and cytochrome c reductase activity. The authors state that their results suggest that P-450 is involved in CCl_4 activation and that irreversible binding of CCl_4 metabolites to cellular components, rather than lipid peroxidation, is responsible for some biochemical and/or ultrastructural lesions reported in different tissues. In another study, $[^{14}C]CCl_4$ has been observed to bind irreversibly to rabbit microsomal proteins at a rate of ~20 nmole/mg protein/hour (Uehleke and Werner, 1975). Binding of CCl_4 (or its metabolites) to hepatic macromolecules was enhanced in the absence of oxygen, consistent with the proposal that the trichloromethyl radical is the reactive metabolite. Furthermore, the order of species susceptibility to liver necrosis from CCl_4 more closely parallels the species order for $[^{14}C]CCl_4$ binding to cellular components than the species order for lipid peroxidation (Diaz Gomez et al., 1975):

Effect	← Most susceptible Least susceptible →
Liver necrosis	mouse > guinea pig = hamster > rat > chicken
$[^{14}C]CCl_4$ binding	mouse = hamster > guinea pig > rat > chicken
Lipid peroxidation	rat > hamster = guinea pig > chicken = mouse

These authors also report that in mice liver necrosis proceeded 24 hours in the absence of lipid peroxidation.

Although it has been shown that $[^{14}C]$ from CCl_4 binds to proteins, the question of CCl_4 binding to polynucleotides remains. The issue is important because of its implications for the mechanism of CCl_4 carcinogenicity and mutagenicity. In the only experiment addressing this question, Uehleke and Werner (1975) incubated $[^{14}C]CCl_4$ with either isolated liver microsomes (rat or mouse, species not identified) or with added soluble RNA. They reported no $[^{14}C]$ binding to ribosomal RNA or exogenous RNA. Experimental details were not presented, but it appears possible to tentatively conclude that proteins -- rather than nucleic acids -- are the main sites of macromolecular CCl_4 binding.

8.3.4. Lipid Peroxidation. A number of the hepatic effects resulting from CCl_4 exposure, including the fatty liver syndrome, are believed to arise as a result of lipid peroxidation (Recknagel and Glende, 1973). The mechanism proposed for the peroxidation is presented below, followed by a discussion of the evidence that this biochemical sequence results in the hepatic lesions associated with CCl_4 poisoning.

The first step in the reaction sequence proposed for lipid peroxidation is production of free radicals, especially the trichloromethyl free radical. The radical initiates a chain reaction by reacting with the hydrogen atom of a $-CH_2-$group in an unsaturated fatty acid, generating a fatty acid free radical. On reaction with molecular oxygen, the fatty acid free radical is converted into an unstable organic peroxide. The peroxide disintegrates in two fashions: (1) intramolecular cyclization to form malonic dialdehyde and two new free radicals, or (2) simple homolytic fission that also yields two free radicals. This whole process occurs autocatalytically: each free radical gives rise to two new free radicals. Figure 8-1 summarizes this hypothesis (Recknagel and Glende, 1973).

A number of indices have been used in in vivo and in vitro assays of lipid peroxidation: pentane and ethane levels in exhaled air (arising from fatty acid decomposition) and malonic dialdehyde concentrations in hepatocytes (arising from intramolecular cyclization). Pentane production in male Sprague-Dawley rats increased by factors of 4.6, 13.2 and 26.4 over that in mineral oil controls within 30 minutes following i.p. administration of CCl_4 doses of 160, 480 and 1440 mg/kg bw, respectively (Sagai and Tappel, 1979).

A mechanism for the pathogenesis of CCl_4-induced hepatic lesions based on lipid peroxidation has been proposed recently (Pasquali-Ronchetti et al., 1980). According to this hypothesis, lipid peroxidation is suggested to affect primarily unsaturated acyl chains of membrane phospholipids, resulting in breakage of the hydrocarbon and loss of phospholipids from the membrane. Lipid peroxidation would therefore produce progressive degenerative changes in the assembly of membranous structures such as (rat) liver endoplasmic reticulum, or its in vitro counterpart, microsomes.

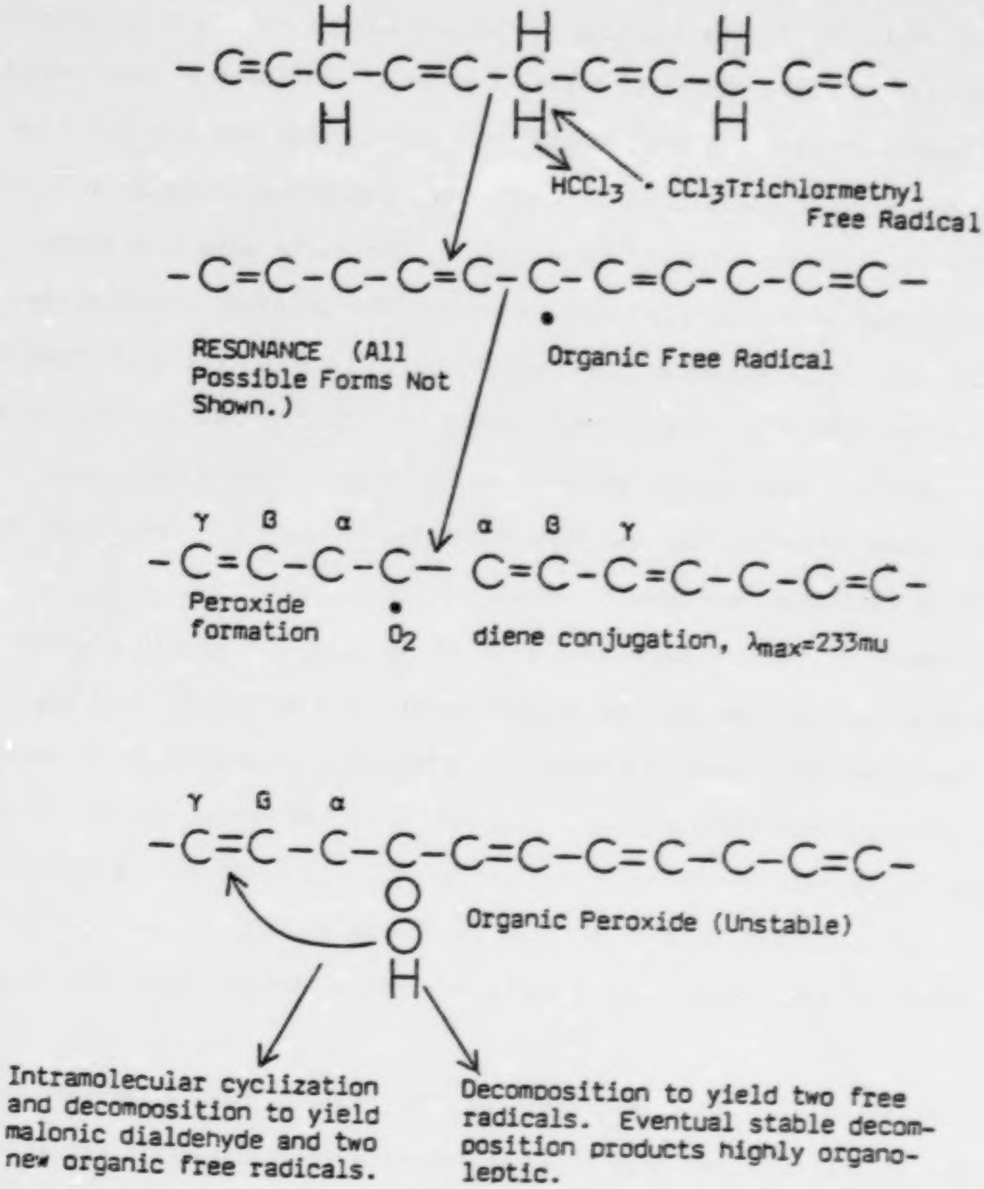

HCCl₃
• CCl₃Trichlormethyl Free Radical
RESONANCE (All Possible Forms Not Shown.)
Organic Free Radical
Peroxide formation
O₂
diene conjugation, λmax=233mu
Organic Peroxide (Unstable)
Intramolecular cyclization and decomposition to yield malonic dialdehyde and two new organic free radicals.
Decomposition to yield two free radicals. Eventual stable decomposition products highly organoleptic.

FIGURE 8-1

Free Radical Initiated, Autocatalytic Peroxidation of

Polyenic Long-Chain Fatty Acids

Source: Adapted from Recknagel and Glende, 1973

This hypothesis is supported by studies showing that treatment with CCl_4 produced lipid peroxidation in male Wistar rat liver endoplasmic reticulum at a concentration of 0.5 mℓ/100 g bw (Pasquali-Ronchette et al., 1980), caused disintegration of endoplasmic reticulum in vitro within 10 minutes at a concentration of 636 mg/ℓ (Pasquali-Ronchetti et al., 1980), and was incorporated predominantly into liver phospholipids in rats (Table 8-7) (Ciccoli and Casini, 1978).

8.4. SUMMARY

Carbon tetrachloride is toxic to humans and animals following inhalation, ingestion or dermal administration. Acute, subchronic and chronic exposures primarily affect the central nervous system, liver and kidneys. Sporadic cases of ocular toxicity also occur following subchronic and chronic exposure to CCl_4 vapor. However, these ocular signs do not correlate with exposure levels or other organ toxicities. Ingestion of alcohol appears to increase susceptibility to CCl_4 toxicity, but the mechanisms are unknown.

8.4.1. Experimental Animal Data. The toxicity of CCl_4 following acute inhalation, ingestion and dermal exposures has been reported for various species. Animals surviving acute doses of CCl_4 developed liver damage and, in some cases, kidney damage. These injuries were dose related.

Subchronic/chronic studies of CCl_4 exposure in rats, monkeys, rabbits, dogs and guinea pigs demonstrated liver, kidney, sciatic nerve, optic nerve and ocular muscle damage.

It has been observed that exposure to a higher concentration over a shorter period of time produces a greater effect upon the liver than exposure to a lower concentration over a longer period of time even though the product of time and concentration is equal in both cases.

TABLE 8-7

Incorporation of ^{14}C from [^{14}C]Carbon Tetrachloride into Lipids of Various Rat Tissues[a,b]

Tissues	Total Lipids (dpm/mg)	Acetone Precipitate (phospholipids) (dpm/mg)	Acetone Supernatant Lipids (dpm/mg)
Liver (6)	112.6 ± 7.4	135.7 ± 14.6	53.3 ± 4.8
Intestinal mucosa	61.8 ± 7.5	69.5 ± 7.7	48.3 ± 8.4
Kidney (6)	23.4 ± 2.6	25.6 ± 3.9	11.4 ± 2.0
Adrenals[c]	8.0	22.0	2.4
Lung (5)	11.3 ± 1.8	15.2 ± 1.4	6.5 ± 0.8
Spleen (6)	8.7 ± 1.2	7.4 ± 2.3	4.0 ± 1.0
Testis (6)	6.3 ± 1.5	5.2 ± 1.4	2.3 ± 1.0
Brain (6)	3.3 ± 0.3	3.7 ± 0.3	0.7 ± 0.2
Heart (6)	2.2 ± 0.8	2.6 ± 1.4	0.8 ± 0.5
Skeletal muscle (6)	0.7 ± 0.1	2.9 ± 0.7	0.6 ± 0.2
Plasma[d]	5.1	trace	3.7

[a] Source: Adapted from Ciccoli and Casini, 1978

[b] [^{14}C]CCl_4 dose: 4000 mg/kg bw (58.6 x 10^6 dpm). Values are expressed as means ± S.E.M. The number of rats is reported in parentheses.

[c] Eight pooled adrenals

[d] Plasma of two animals

dpm = disintegrations per minute

It is recognized that interspecies differences exist particularly with regard to the metabolism of CCl_4 and other pharmacokinetic parameters as well as sensitivity to CCl_4. However, data quantifying such differences have not been found in the available literature. Methods of dose conversion from animal species to humans are discussed in the Appendix.

8.4.2. Human Data. Considerable human exposure to CCl_4 through inhalation has occurred through its use as an industrial solvent and dry cleaning fluid. Ingestion of CCl_4 or of mixtures containing CCl_4 has also been documented in various case reports. Ingestion has occurred under different circumstances by persons of diverse occupations and ages. These acute exposures have been followed by hepatoxic effects accompanied by acute nephrosis.

Hepatic necrosis and renal pathology appear to be characteristic effects of acute human exposure to CCl_4. If exposure is terminated, the liver shows regeneration in most cases. In cases of acute renal dysfunction, kidney function returns to normal after exposure is terminated and medical treatment is given.

In many of the case reports and older studies, the investigators present the data in narrative form. Although interesting, these type of data are not suitable to quantitative analysis since numbers are not adequately presented. Furthermore, there usually are a number of uncontrolled variables (alcohol intake, age, simultaneous exposures) or unknown variables (exposure amount) making it difficult to attribute the outcome solely to the CCl_4 exposure. The experimental human studies are not numerous, yet they are important since they can either support or challenge the experimental animal studies and can aid qualitatively in the extrapolations from animals to humans.

8.4.3. Mechanisms of Toxicity. The chemical pathology of CCl_4 liver injury is generally viewed as an example of lethal cleavage, where the CCl_3-Cl bond is split in the mixed function oxidase system of hepatocytes. Two major sequences of this cleavage have been suggested; both views presume the formation of free radicals from the homolytic cleavage of the CCl_3-Cl bond (i.e., $\cdot CCl_3$ and $\cdot Cl$). One sequence entails the direct attack (via alkylation) by free radicals on cellular constituents, notably protein sulfhydryl groups. The second sequence involves the extraction of a hydrogen atom by the trichloromethyl free radical from a long-chain fatty acid to form chloroform and a fatty acid free radical. Molecular oxygen, because of its triplet ground state, binds with the unpaired electron on the fatty acid radical to form an organic peroxide. The peroxide is unstable and decomposes to form more organic free radicals, which in turn form more organic peroxides (Recknagel and Glende, 1973). This process appears to lead to fatty acid chain decomposition, with the resulting breakdown of membrane structure (Recknagel and Glende, 1973). This breakdown may lead to a halt in lipid excretion via the Golgi apparatus, with fatty liver occurring as a consequence. Cell necrosis would also follow directly from lipid destruction. The mechanism by which lipid peroxidation could lead to cell transformation is not explained at present, and the molecular events leading to CCl_4 carcinogenicity remain unknown.

In addition to these proposed mechanisms of toxicity, two minor metabolic pathways have been postulated: dimerization of two trichloromethyl free radicals to form hexachloroethane (Fowler, 1969) and the formation of a trichloromethyl peroxy radical which may result in production of phosgene and carbon dioxide (Shah et al., 1979). Both hexachloroethane and phosgene are toxic, but the extent of their contribution to observed hepatotoxicity is unknown. Carbon tetrachloride has not been found to bind to cellular polynucleotides, but presently only one investigation studying the binding of CCl_4 to such nucleotides has been reported (Uehleke and Werner, 1975).

9. TERATOGENICITY AND OTHER REPRODUCTIVE EFFECTS

9.1. TERATOGENICITY

In two inhalation studies on the teratogenic and prenatal toxicologic effects of CCl_4, the chemical was reported to produce prenatal toxicity but not teratogenicity. Schwetz et al. (1974) exposed pregnant Sprague-Dawley rats to CCl_4 at 1800 or 6300 mg/m³ for 7 hrs/day on days 6-15 of gestation. Statistically significant decreases in fetal body weight and crown-rump length were observed. Other parameters examined such as sex ratio, live fetuses/litter and resorptions were not significantly different from those of controls. Two other statistically significant fetal effects were noted: an increased incidence of litters with sternebral anomalies in the 6300 mg/m³ group and an increased incidence of litters with subcutaneous edemas in the 1800 mg/m³ group. The incidence of litters with edema in the 6300 mg/m³ group (50%), although apparently increased, was not significantly different from the control incidence (33%). The dams exposed to both concentrations of CCl_4 showed a decreased food consumption compared to control animals and a statistically significant decrease in weight gain. Both of these effects were greater at the higher dose level. Hepatotoxicity, as measured by significantly increased SGPT activity, was also seen in the dams following daily exposure to 1800 or 6300 mg/m³ CCl_4 but the increase was greater at the lower dose level. No consistent pattern was established between fetal toxicity and maternal toxicity at the subanesthetic levels of CCl_4 used in this experiment. The authors concluded that CCl_4 was not highly embryotoxic at the concentrations used. The evidence of maternal toxicity precluded any statement about the teratogenic potential of CCl_4.

The other inhalation study resulted in no teratogenic effects following exposure of pregnant rats to CCl_4 at 1575 mg/m³ 8 hrs/day for 5 consecutive days between days 10-15 of pregnancy (Gilman, 1971). Concomitant exposure to 15% ethanol in drinking water also did not result in teratogenic effects. Carbon tetrachloride exposure, however, did decrease the viability index to 83% as compared to 99% for controls ($p<0.1$), resulting in a decrease in the number of pups per litter, 9.2 as compared to 10.3 for controls. The lactation index was also decreased to 83% compared to 98% for controls. Concomitant ethanol exposure exacerbated the former effect: 8.48 pups per litter compared to 10.3 for controls. Although only 10 control and 25 experimental animals were used, thus lessening the sensitivity, the results of this study indicate prenatal toxicity and tend to support those of Schwetz et al. (1974).

Subcutaneous exposure of CCl_4 to pregnant rats has resulted in liver damage in fetuses and neonates (Bhattacharyya, 1965). Administration of 1600 mg/kg CCl_4 s.c. on days 20 or 19 of gestation resulted in small areas of focal hepatic necrosis in neonates born 48 or 72 hours later, respectively. Histologic findings generally included a sharply demarcated area of centrilobular necrosis and proliferation changes in nonnecrotic lobes.

The investigator also treated fetuses directly with CCl_4 by subjecting the dam to a laparotomy and either injecting the chemical directly into the fetus or into the amniotic sac through the uterine wall. Liver changes following injection of 6 mg CCl_4 were variable; cells generally became extremely pale in centrilobular and midzonal areas, indicating fatty infiltration. Livers remained abnormal until at least 4 days after birth. No necrosis, hemorrhage or regeneration was observed.

Liver sensitivity of neonate rats to CCl_4 was reported to be low 1 hour after birth, then to rise above the adult level at 19 hours and to decline to adult levels by 3-7 days after birth. Thus, only 2 of 10 1-hour-old neonates receiving 1600 mg/kg CCl_4 s.c. showed centrilobular necrosis after 24 hours. In addition, hepatic portal areas contained numerous neutrophils, but in contrast to findings in adult animals, no bile duct proliferation could be observed. More pronounced hepatic damage was found in 19-hour neonates than 1-hour neonates. Damage declined in 3- and 4-day-old neonates; that in 5-, 6- and 7-day-olds was similar in appearance to that of adults.

Neonates can apparently be exposed to CCl_4 through mothers' milk (Bhattacharyya, 1965). Subcutaneous administration of CCl_4 at 1600 or 3200 mg/kg bw to four nursing rats resulted in hepatic damage in the neonates 24 or 48 hours later. A dose of 800 mg/kg bw to dams did not produce hepatic damage to offspring. Levels in the milk were not reported.

The pre- and postnatal toxicologic studies described above do not meet current design criteria for hazard assessment purposes in that fewer than three dose levels were used and a second, nonrodent species was not studied (U.S. EPA, 1981). Furthermore, positive controls were not used. No study of specific postnatal functional lesions was available.

9.2. OTHER REPRODUCTIVE EFFECTS

Testicular degeneration was observed in rats receiving CCl_4 at 4800 mg/kg bw i.p. (Chatterjee, 1966). One group of six male rats received CCl_4 as a 1:1 mixture in coconut oil. The vehicle control group received only an equal volume of coconut oil. All animals were sacrificed on day 15. Body weights were similar for treated and control animals. However, the relative testes weight decreased from 15.5 (±0.4) g/kg bw in controls to 9.8

(±1.2) g/kg bw in exposed animals. A decrease in testis size and weight is a good indicator of a decline in male spermatogenic process. Relative weight of seminal vesicles showed an even more pronounced decrease: 1.27 (±0.171) g/kg bw in treated as compared to 3.10 (±0.059) g/kg bw in control animals. Relative pituitary weight, however, increased: 50.0 (±1.4) mg/kg bw in treated as compared to 32.4 (±0.9) mg/kg bw in control animals. This increase would be expected if the feedback mechanism from the testis was obliterated and pituitary concentrations of gonadotrophin were increased. However, in this study actual pituitary content of gonadotrophins was not measured. Therefore, no conclusions can be made on the effect of the chemical on the feedback mechanism, making the significance of the increase in pituitary weights difficult to interpret.

Further, histological examination of testes in CCl_4-treated animals showed testicular atrophy and "some abnormality" in spermatogenesis. The author proposed a mechanism for CCl_4-induced testicular atrophy in which blockage of pituitary hormone release results in atrophy of Leydig cells within the seminal vesicles, followed by an abnormal spermatogenesis.

In another study (Kalla and Bansal, 1975), intraperitoneal administration of CCl_4 (4800 mg/kg bw as a 1:1 mixture of coconut oil) to male rats for 10, 15 or 20 days (Group I, II or III, respectively) led to impairments in spermatogenesis as indicated by histological examination. Vehicle controls were administered equal volumes of coconut oil. Weights of testes, seminal vesicles, epididymis and prostates were decreased in exposed animals, whereas the weight of adrenals increased (Table 9-1). The gonadosomatic index (GSI: equal to body weight x testes weight/100) was also decreased in treated animals. A slight decrease in pituitary weight was observed following a 10-day treatment but not after either the 15- or 20-day

TABLE 9-1

Weight Changes in Male Rat Reproductive Organs After Carbon Tetrachloride Treatment[a,b] (4800 mg/kg bw i.p. as a 1:1 mixture of coconut oil)

Treatment and Period	Body Weight (g)		Testis (g/kg bw)	Seminal Vesicles (g/kg bw)	Epididymis (g/kg bw)	Prostate (g/kg bw)	Adrenal (g/kg bw)	GSI[c]
	Before Treatment	After Treatment						
Group I 10 days								
Control	257± 5.0	269± 4.5	12.36±0.94	4.25±0.55	4.45±0.45	2.1 ±0.7	0.18±0.02	7.94±0.45
Treated	257±13.71	247±10.76	8.84±1.04	2.41±0.14	2.88±0.30	1.26±0.24	0.24±0.03	5.68±0.31
Group II 15 days								
Control	230±15.49	235± 5.0	10.47±0.59	3.9±0.52	4.12±0.18	2.19±0.31	0.22±0.01	6.02±0.21
Treated	230±15.49	173±13.42	9.46±0.33	1.17±0.26	2.63±0.18	1.68±0.13	0.32±0.05	5.44±0.28
Group III 20 days								
Control	234± 5.5	235± 6.5	11.37±0.06	3.24±0.31	3.72±0.09	1.78±0.04	0.12±0.01	6.64±0.23
Treated	230± 3.5	219± 0.5	9.9 ±0.65	1.53±0.28	2.52±0.22	1.29±0.01	0.25±0.02	5.69±0.38

[a] Source: Adapted from Kalla and Bansal, 1975

[b] Mean ± standard deviation

[c] Gonadosomatic index = body weight x testis weight/100

treatment. As reported, the ratio of germinal to nongerminal area steadily decreased from Group I to Group III and was always higher in treated than control animals. Significant differences in total germinal area between treated and control animals, however, were observed only at 20 days. Histological examination did not reveal any abnormalities in testes from Group I. Clusters of mature sperm were present in the lumen. In Group II, slight testicular damage was observed: a decrease in spermatogenic cells and increased lumen size. In Group III, shrinkage of the tubules and increased area of the lumen were observed. Arrangement of the germ cells was disrupted; early gonadal cells were present in the lumen of many of the tubules. No spermatids were observed. Interstitial material was "damaged" and in many places the basement membrane was detached from the epithelium.

Problems associated with this study include: that the authors do not report numbers of animals studied at each dose level; the authors do not report the frequency of treatments; the authors do not report whether the dose of 3 mℓ/kg bw was the total dose or the dose given at each treatment, although the former is assumed. Thus, noting the ambiguity in the reporting of dose levels, CCl_4 at an assumed total dose of 3 mℓ/kg bw over 10 days had a distinct but minor effect on male rat reproductive physiology, whereas a total dose of 96 g/kg bw over 20 days resulted in severe disturbances of spermatogenesis. In view of the increased pituitary weight and the decreased adrenal weight, a possible mechanism of action of CCl_4's male reproductive effect could be the suppression of pituitary gonadal axes. However, as pointed out earlier in this section, pituitary weight by itself is almost a meaningless parameter. Many hormones are located in the total pituitary, and reproductive hormones, such as gonadotrophins, are located only in the anterior pituitary. Before a conclusion can be made about pituitary content of various hormones, measurements of the synthesized, stored and released hormones should be made. In addition to these criticisms, the two studies described in this section had shortcomings with regard to current experimental design criteria for hazard assessment: less than three dose levels and no data for a second, nonrodent, species.

The relationship between maternal toxicity and reproductive effects was investigated by Wilson (1954). The author compared fetal and maternal toxicities of rats (strain not given) across several treatments and concluded that not all physiological disturbances of the mother will alter fetal development. In fact, little correlation existed across treatments between the severity of maternal toxicity and the extent of effect to the surviving offspring. In the treatment consisting of CCl_4 poisoning, surviving litters were virtually normal, even though several dams died and others had premature termination of pregnancy (i.e., resorption). The exposure schedule was 0.3 cc CCl_4 by stomach tube or 0.8 cc subcutaneously in corn oil given 2 or 3 times on as many successive days during gestation.

Teratogenic effects in humans caused by CCl_4 exposure have not been reported. However, human fetuses in one study appeared to have selectively accumulated CCl_4 from the mothers' circulation (Dowty and Laseter, 1976). Maternal blood samples were taken from 11 women either before or directly after vaginal delivery (prior exposure of the women to toxic chemicals was not reported). Paired cord blood samples were obtained immediately after delivery. All volatiles were analyzed by gas chromatography and mass spectrometry. Carbon tetrachloride, benzene and chloroform were present in higher concentrations in cord blood as compared to maternal blood.

9.3. SUMMARY

Carbon tetrachloride has produced prenatal toxic effects, some of which (i.e., subcutaneous edema) could not be associated with extent of maternal exposure. Rats exposed to CCl_4 in utero have shown hepatic abnormalities at birth, but the fetal rat liver appears to be less sensitive than the adult liver to the hepatotoxic effects of CCl_4.

Carbon tetrachloride has produced distinct degenerative changes in testicular histology in rats, eventually resulting in aspermatogenesis and functional male infertility. These effects occurred following intraperitoneal injection at relatively high doses. Unfortunately, low doses were not tested.

Due to the limited number and scope of the studies reported in this chapter, it is difficult to evaluate the potential of CCl_4 to cause adverse teratogenic, embryotoxic or reproductive effects. Some of the specific limitations are provided within the discussion of each study. In general, the studies do not provide adequate dose groups for concluding the existence of teratogenic or reproductive effects according to testing criteria such as those currently used for U.S. EPA Office of Pesticides Programs or Office of Toxic Substances.

10. MUTAGENICITY

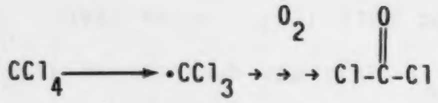
The mutagenic potential of CCl_4 has been assessed by evaluating the results of seven bacterial studies, one yeast study, one in vitro mammalian chromosome damage study and three in vivo DNA damage studies in rodents. The majority of these studies were negative. Information relating to the metabolism of CCl_4 and to covalent binding of the metabolites to cellular macromolecules (including DNA) is presented before the sections assessing the genotoxicity of CCl_4. This was done to set the stage for the discussion of the largely negative results obtained in the mutagenicity studies described below and for the suggestion that CCl_4 may be a weak mutagen. In addition, suggestions for additional testing are presented.

10.1. METABOLISM AND COVALENT BINDING TO MACROMOLECULES

The evidence described in this section suggests that CCl_4 is metabolized in the liver to highly reactive intermediates (the trichloromethyl free radical and phosgene). The evidence also indicates that metabolically activated CCl_4 covalently binds to protein, lipid and DNA, suggesting that CCl_4 may have genotoxic potential.

10.1.1. Metabolism. Carbon tetrachloride is metabolized in the liver endoplasmic reticulum by the cytochrome P-450 component of the mixed function oxidase system (Reynolds and Moslen, 1980). The available evidence indicates that metabolism of CCl_4 results in the generation of the trichloromethyl free radical $\cdot CCl_3$ (Reynolds and Moslen, 1980; Trudell et al., 1982) and phosgene (Shah et al., 1979; Kubic and Anders, 1980; Pohl et al., 1981). These two substances are the most likely of the metabolites to exhibit reaction with tissue macromolecules because of their high reactivity. By using human cytochrome P-450 reconstituted in phospholipid vesicles, Trudell et al. (1982) have demonstrated that $\cdot CCl_3$ is the major product of the reductive metabolism of CCl_4 as determined by mass spectral identification of the adducts formed between $\cdot CCl_3$ and the phospholipid dioleoyl phosphatidylcholine.

Phosgene results from further metabolism of $\cdot CCl_3$ (Shah et al., 1979) (see Figure 7-1).



$$CCl_4 \xrightarrow{O_2} \cdot CCl_3 \rightarrow \rightarrow \rightarrow Cl-\overset{\overset{O}{\|}}{C}-Cl$$

Pohl et al. (1981) measured the amount of phosgene (as diglutathionyl dithiocarbonate) produced from the aerobic metabolism of CCl_4, $CHCl_3$ and $CBrCl_3$ by liver microsomes (from phenobarbital-treated rats) plus cofactors. The results indicate that phosgene production from CCl_4 is only 4% of that produced from $CHCl_3$. Thus, the level of phosgene production from aerobic metabolism of CCl_4 is small. The carcinogenicity and DNA binding of phosgene are currently under investigation in Dr. B.L. Van Duuren's laboratory at New York University Medical Center. Preliminary evidence indicates that phosgene binds to DNA (*Dr. Sipra Banerjee, personal communication*). Metabolites of CCl_4 are so reactive that they bind to and inactivate the cytochrome P-450 enzymes that were responsible for their generation (suicide mechanism) (Vainio et al., 1976; Sipes et al., 1977; De Groot and Haas, 1980; Cooper and Witmer, 1982).

10.1.2. Covalent Binding to Macromolecules. Metabolically activated CCl_4 has been found to bind covalently* to lipid and protein both *in vivo* (Rocchi et al., 1973; Diaz Gomez and Castro, 1980a,b; and *in vitro* (Rocchi et al., 1973; Uehleke et al., 1977). Uehleke et al. (1977) measured covalent binding of 1 mM $[^{14}C]CCl_4$ (0.25 Ci/mol) to microsomal protein and lipid in liver microsome suspensions (5 mg protein/mℓ plus cofactors) from phenobarbital-treated rabbits. About 10% of the ^{14}C label was covalently bound to endoplasmic reticulum protein and >30% was bound to microsomal lipid. Extramicrosomal binding was evaluated by the addition of 5 mg of bovine serum albumin per mℓ to the CCl_4/microsome mixture. The binding of metabolically activated $[^{14}C]CCl_4$ to added bovine serum albumin (1.4 nmol/mg in 60 minutes) was about 1.5% of that bound to microsomal protein (20.0 nmol/mg) plus lipid (76.0 nmol/mg). Thus, it appears that binding of metabolically activated $[^{14}C]CCl_4$ to extramicrosomal macromolecules is negligible compared to binding to microsomal constituents. (Mutagenicity studies were also described in this paper and are discussed in the next section.)

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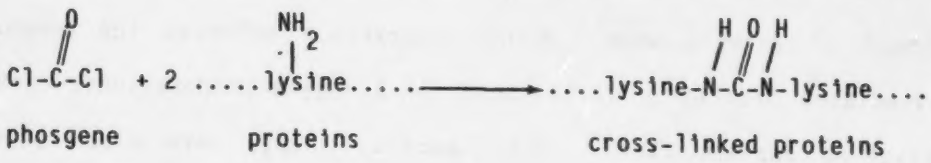
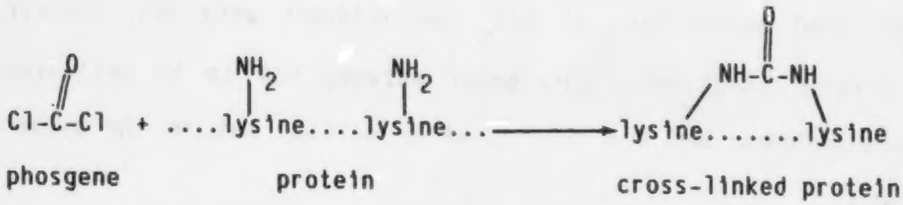
*Amount of label bound was determined after washing and extraction, indicating that the binding was covalent.

Evidence that the CCl_4 metabolite, phosgene, reacts with proteins was obtained by Cessi et al. (1966) when they measured the in vivo binding of CCl_4 to rat liver proteins and compared it to the in vitro acylation of poly-L-lysine and serum albumin by phosgene. Similar reaction products were obtained in both systems, suggesting that phosgene reacts with the lysine ε-amino groups in proteins, leading to cross-linked carbonyl derivatives:



$$\underset{\text{phosgene}}{\mathrm{Cl{-}\overset{\overset{O}{\|}}{C}{-}Cl}} + 2\ \underset{\text{proteins}}{\ldots\ldots\overset{\overset{NH_2}{|}}{\mathrm{lysine}}\ldots\ldots} \longrightarrow \underset{\text{cross-linked proteins}}{\ldots\mathrm{lysine{-}\overset{H}{N}{-}\overset{\overset{O}{\|}}{C}{-}\overset{H}{N}{-}lysine}\ldots}$$

or



$$\underset{\text{phosgene}}{\mathrm{Cl{-}\overset{\overset{O}{\|}}{C}{-}Cl}} + \underset{\text{protein}}{\ldots\overset{\overset{NH_2}{|}}{\mathrm{lysine}}\ldots\overset{\overset{NH_2}{|}}{\mathrm{lysine}}\ldots} \longrightarrow \underset{\text{cross-linked protein}}{\mathrm{lysine}\overset{\mathrm{NH{-}\overset{\overset{O}{\|}}{C}{-}NH}}{\ldots\ldots\ldots}\mathrm{lysine}}$$

Such cross-linked proteins would undoubtedly exhibit impaired biological activity. It is also possible that similar cross-linking reactions of phosgene can occur with amino groups in DNA, resulting in alterations in DNA structure and function.

Binding of metabolically activated CCl_4 to DNA was found by two groups. Rocchi et al. (1973) studied the binding of CCl_4 to nucleic acids and protein. ^{14}C-labeled CCl_4 (367 µmol/kg) was injected into rats and mice and the amount of metabolite(s) of CCl_4 that covalently bound to liver DNA, RNA, nuclear proteins, and cytoplasmic proteins was measured. Significant amounts of labeled material were found associated with rRNA, nuclear proteins and cytoplasmic proteins in rats. When the rats were pretreated with 3-methylcholanthrene (5 mg, 24 hours before treatment with CCl_4), the amount of label associated with the macromolecules increased. No label was associated with DNA in the rat studies. Similar studies in mice indicated that DNA binding occurred (108 µmol CCl_4/mol DNA), but only after pretreatment with 3-methylcholanthrene (1 mg, 24 hours before CCl_4 dosing).

In an in vitro experiment, Rocchi et al. (1973) used rat or mouse liver microsomes to activate labeled CCl_4 in the presence of calf thymus DNA. Pretreatment of animals with 3-methylcholanthrene enhanced the amount of label associated with DNA. Furthermore, pH 5 enzyme preparations, containing activating enzymes (Keller and Zamecnik, 1956), were also found to increase the amount of label bound to DNA. Therefore, from these results, it appears that metabolites of CCl_4 can interact with DNA; however, for optimal binding conditions, microsomal enzymes had to be activated with 3-methylcholanthrene and the binding assay carried out in the presence of pH 5 enzymes.

Diaz Gomez and Castro (1980a) have also measured binding of metabolically activated CCl_4 to cellular macromolecules. The results show that ^{14}C from $[^{14}C]CCl_4$ (specific activity, 27 Ci/mol) irreversibly bound in vivo to liver nuclear DNA, protein and lipids in strain A/J mice and Sprague-Dawley male rats. Mouse and rat liver nuclear DNA isolated from animals given $[^{14}C]CCl_4$ 16 hours before they were killed exhibited a small but significant labeling (mice, 0.72 pmol/mg; rats, 0.52 pmol/mg). The count from the assay done in the presence of unlabeled DNA was subtracted from the experimental counts before binding was calculated. In contrast to the results of Rocchi et al. (1973), induction of liver enzymes by 3-methylcholanthrene was not required for binding of ^{14}C from $[^{14}C]CCl_4$ to DNA. It should be mentioned that the purified DNA samples contained 0.2% protein. However, contamination by protein at this low level could not account for all the covalent binding measured in the DNA sample.

Significant in vitro binding of metabolically activated CCl_4 to isolated mouse liver DNA (1.81 pmol/mg) was observed by Diaz Gomez and Castro (1980a) in anaerobic incubation mixtures containing microsomes and NADPH. It was also found that $\cdot CCl_3$ chemically produced by reaction of CCl_4 with benzoyl peroxide interacted with DNA (826 pmol/mg). This result suggests that $\cdot CCl_3$ may be the species involved in the binding to DNA.

In addition to the above observations that metabolites of CCl_4 bind to DNA, Diaz Gomez and Castro (1980a) also observed significant binding in vivo of metabolically activated CCl_4 to rat liver nuclear protein and lipid. The label bound to nuclear protein was 47.7 pmol/mg and that bound to nuclear lipid was 113.5 pmol/mg. The authors suggested that the binding of metabolically activated CCl_4 to nuclear lipids is significant in terms of the carcinogenicity of CCl_4, because nuclear lipid is derived from the nuclear membrane which contains the P-450 necessary for activation of CCl_4 to the probable reactive metabolites $\cdot CCl_3$ and phosgene. Since these metabolites are highly unstable and therefore are not likely to exist long enough to travel from the endoplasmic reticulum P-450 enzymes to nuclear components, activation by nuclear membrane P-450 enzymes is more likely to yield interactions of the metabolites with nuclear components (e.g., DNA) than will activation in the endoplasmic reticulum.

Diaz Gomez and Castro (1980b) have assessed the CCl_4 activation potential of purified rat liver nuclei by measuring covalent binding of nuclear-activated CCl_4 to nuclear protein and lipid. Binding to DNA was not measured in this study. The results were compared to results obtained from similar incubation mixtures containing microsomes instead of purified nuclei. The incubation mixtures containing either nuclei (1.3 mg protein/mℓ) or microsomes (1.56 mg protein/mℓ) were incubated for 30 minutes in 37.6 nM $[^{14}C]CCl_4$ (6.94 Ci/mol) and an NADPH generating system in an O_2-free N_2 atmosphere.

The authors observed that the extent of binding to proteins in the nuclear preparations was 43.5% of that observed for microsomes (nuclear suspensions, 21.9 pmol/mg; microsomes, 50.3 pmol/mg). Binding to nuclear lipids was 77.3% of that observed for microsomes (nuclear suspension, 147 pmol/mg; microsomes, 190 pmol/mg). Thus, isolated nuclei were less efficient than microsomes in metabolizing CCl_4 but the results were within the same order of magnitude.

This study suggests that metabolism of CCl_4 to reactive intermediates can occur in nuclear membranes and indicates that the *in vivo* binding observed in the previous study (Diaz Gomez and Castro, 1980a) may have been due to nuclear rather than microsomal activation of CCl_4. It should be mentioned, however, that the nuclear preparations were contaminated with trace amounts of endoplasmic reticulum, which may have been sufficient to result in at least part of the nuclear activation observed.

Diaz Gomez and Castro (1981) have published preliminary evidence that $\cdot CCl_3$, chemically generated from the benzoyl peroxide-catalyzed decomposition of CCl_4, reacts primarily with guanine and adenine and to a lesser extent with cytosine and thymine. This result suggests that $\cdot CCl_3$ may bind to DNA _in vivo_ by interaction with the deoxyribonucleic acid bases.

In summary, it has been shown that CCl_4 is metabolized to reactive intermediates ($\cdot CCl_3$ and phosgene). It has also been shown that metabolically activated CCl_4 binds to DNA, protein and lipid. These results suggest that CCl_4 has genotoxic potential. The negative results in six of the seven bacterial mutagenicity studies described in the next section may be due to inadequate metabolic activation in the test systems used or may result from the scavenging by protein or lipid of any very reactive metabolic intermediates formed (e.g., $\cdot CCl_3$ and phosgene) under conditions of exogenous activation, thus limiting their availability for reaction with DNA. For these reasons, test systems used to assess the genotoxicity of CCl_4 should incorporate adequate metabolic activation and an indication that any highly reactive metabolites formed were not scavenged by microsomal protein or lipid before reaching the DNA of the test organism.

10.2. MUTAGENICITY STUDIES IN BACTERIAL TEST SYSTEMS

Studies to determine the mutagenic activity of CCl_4 in the _Salmonella typhimurium_ revertant system have been primarily negative. A review article written by McCann et al. (1975) stated that an assay using Aroclor-induced S9 activation and strains TA100 and TA1535 was negative. This article contained no details of the procedure used to generate this negative result.

Another review article (Fishbein, 1976) in which no data were presented contained a statement that CCl_4 was not mutagenic when assayed in a spot test with the TA1950 strain.

Uehleke et al. (1977) tested the mutagenicity of CCl_4 in suspension assays with S. typhimurium strains TA1535 and TA1538. No mutagenic activity was observed. About 6-9x10^8 bacteria were incubated for 60 minutes under N_2 in tightly closed test tubes with 8 mM CCl_4 and microsomes (5 mg protein) plus cofactors. The mutation frequencies (his$^+$ colony forming units/10^8 his$^-$ colony forming units) were <10 for both strains and the spontaneous mutation frequencies were 3.9±3.7 for strain TA1535 and 4.4±3.5 for strain TA1538. At this concentration of CCl_4, survival of the bacteria was at least 90%. Thus, the negative results for CCl_4 cannot be interpreted since concentrations of CCl_4 resulting in <90% survival should also have been tested. Dimethylnitrosamine, cyclophosphamide, 3-methylcholanthrene and benzo(a)pyrene were the positive controls used in this study. Although these chemicals were mutagenic in the presence of the S9 activation system, they may not be appropriate as controls for CCl_4 because they are not halogenated alkanes and are, therefore, not metabolized like them.

Because metabolically activated CCl_4 did not significantly bind to exogenously added bovine serum albumin (see binding studies above), Uehleke et al. (1977) concluded that any reactive species generated by the microsomes may not have distributed into the incubation medium and, thus, may have been inaccessible to the test bacteria, resulting in the negative mutagenicity response observed. However, it is not clear from the description provided in this report whether rat, mouse or rabbit microsomes were used in the mutagenicity studies. It is clearly stated that rabbit microsomes were used for the binding studies described above. If mouse or rat microsomes rather than rabbit microsomes were used for the mutagenicity experiments, it cannot be assumed that CCl_4 was sufficiently activated, since activation sufficient for binding of $[^{14}C]CCl_4$ to macromolecules was shown in this paper only with rabbit microsomes. Another deficiency in this study is that the Ames strains TA98 and TA100 were not used. These strains contain an R factor plasmid that increases the sensitivity of the tester strains to certain mutagens. Because of these deficiencies, the negative mutagenicity results in this paper are judged to be inconclusive.

The mutagenicity of CCl_4 was also tested in a study designed to evaluate the mutagenic potential of chemicals identified in drinking water (Simmon et al., 1977). No mutagenic activity was detected with CCl_4. The authors tested 71 of the 300 chemicals that had been identified in public water supplies. Carbon tetrachloride was tested in this study in a desiccator to assess mutagenicity due to vapor exposure and to avoid excessive loss of CCl_4 to the atmosphere. The desiccator contained a magnetic stirrer which acted as a fan to aid in evaporation of the measured amount of CCl_4 and to maintain an even distribution of the vapors. Plates were exposed to the vapors for 7-10 hours and then removed from the desiccators, covered, and incubated ~40 hours before scoring. Mutagenic activity was not observed and no information on toxicity was provided.

This study by Simmon et al. (1977), although lacking some specific details of the CCl_4 assay, clearly identifies certain trihalomethanes ($CHBr_3$, $CHBr_2Cl$, $CHBrCl_2$) as mutagens in the vapor assay in desiccators. Methyl bromide, methyl chloride, methyl iodide and methylene chloride were also found to be mutagenic in the desiccator assay. However, these seven halogenated compounds did not require metabolic activation to exhibit mutagenic activity. It may be that CCl_4 itself is not mutagenic and the rat liver S9 does not effectively metabolize CCl_4 to a potential mutagenic reactive intermediate (? $\cdot CCl_3$), even though the demonstration of mutagenicity of three of the chemicals tested [bis(2-chloroisopropyl)-ether, vinyl chloride and vinylidene chloride] required or was enhanced by this S9 mix. It may also be that a reactive intermediate was formed, but it was too reactive or short-lived to be detected in a test system that uses exogenous metabolic activation.

Another negative result for the mutagenicity of CCl_4 was obtained in a recent study using the Salmonella/microsome assay in which escape of the volatile compounds was prevented by the use of a specially designed, closed, inert incubation system (Barber et al., 1981). Seven of the 10 halogenated alkane solvents tested gave positive mutagenicity results when the Ames assays were carried out in the closed incubation system. Under standard conditions (in which volatilization was not prevented), only 2 of the 10 solvents tested gave a positive result. Thus, the closed incubation system allowed for the detection of five more mutagens than could be detected under standard conditions. Carbon tetrachloride was one of the three solvents tested that gave a negative result in both the standard and closed incubation systems. The investigators indicated that CCl_4 was tested at concentrations high enough to produce observable toxicity (determined by absence of background lawn). The Salmonella strains used were TA1535, TA1537, TA1538, TA98 and TA100. Levels of CCl_4 tested were 4.7, 5.7, 10.2, 12.3 and 18.4 μmol per plate. No dose-related response was observed. The seven solvents that tested positive in this closed system did not require metabolic activation by S9 mix prepared from Aroclor-induced rats (the S9 mix did activate the positive control 2-aminoanthracene). It is possible that the S9 mix used, although adequate for activation of 2-aminoanthracene, was not adequate to metabolize the other three solvents (including CCl_4).

It is also possible that active metabolites, if formed, reacted with microsomal components (see below) or bacterial membrane macromolecules before reaching the bacterial DNA.

In a recent abstract, Cooper and Witmer (1982) reported that exposure of Salmonella strain TA100 to CCl_4 for 20 minutes in a 1 mℓ liquid suspension at low oxygen tension (3 mℓ vacutainer tubes) before preparation of plates resulted in a 2-fold increase in revertants above background (background, 72±9 revertants; 5 μmol CCl_4, 141±20 revertants). Evidence for a dose-response relationship was not reported in this abstract. The weak mutagenic result was observed in two separate experiments and only when fresh rabbit liver S9 was used as the activation system. It is unlikely that the rabbit liver S9 alone was responsible for the mutagenic activity observed because plate assays with rabbit S9 exhibited no mutagenic activity. The CCl_4 was spectrophotometric grade (Spectrar) purchased from Mallinckrodt (Dr. Charlotte Witmer, personal communication). Carbon tetrachloride was toxic in this suspension assay; 2 μmol/mℓ caused 80% toxicity with microsomal activation and 20% without activation. Addition of 0.1 mM EDTA to inhibit microsomal lipid peroxidation decreased bacterial toxicity.

The authors concluded that the suspension assay in the vacutainer tubes may be a suitable system for testing volatile compounds that undergo a reductive metabolism. This weak positive result constitutes preliminary evidence that CCl_4 may be mutagenic in the Ames assay, but only under certain assay conditions (low oxygen tension, exposure of bacteria in suspension in the presence of EDTA, and use of fresh rabbit liver S9).

In summary the results from the above bacterial studies are inconclusive, because false negative results could have been obtained due to a number of factors, including:

1. The activation systems used may have been inadequate for metabolism of CCl_4.

2. $\cdot CCl_3$ and phosgene, the primary reactive metabolites of CCl_4, are unstable and highly reactive. Because exogenous activation systems were used in many of these studies, any $\cdot CCl_3$ or phosgene generated (assuming an adequate activation system) may have been scavenged by microsomal protein or lipid before reaching the DNA.

3. Adequate exposure to CCl_4 may not have occurred if appropriate precautions were not taken to prevent the evaporation of CCl_4.

10.3. STUDIES IN EUCARYOTIC TEST SYSTEMS

Callen et al. (1980) carried out a study on the mutagenicity of CCl_4 in the D7 strain of Saccharomyces cerevisiae, which contains an endogenous cytochrome P-450 dependent monooxygenase activation system. By using this strain of yeast, Callen and his coworkers eliminated the need for the exogenous type of metabolic activation system used in the above bacterial studies. Three different genetic endpoints can be examined with this system: gene conversion at the trp5 locus, mitotic crossing over at the ade2 locus, and gene reversion at the ilv1 locus. The effect of CCl_4 (Mallinckrodt, 99+% pure) on these endpoints was measured by exposing cells in suspension to 3.23, 4.31 and 5.13 g of CCl_4 per liter of buffer (21, 28 and 34 mM, respectively), well above the solubility level of CCl_4 in water (0.8 g/ℓ at 25°C). Therefore, a dose-response relationship could not be obtained because the dose was essentially the same in all cases -- the solubility level of CCl_4 in water at 37°C. Escape of volatilized CCl_4 is not expected to have occurred to any significant extent, because the incubations were carried out in screw-capped glass tubes. Although the dose is essentially co[illegible], amounts in suspension will vary. Extracellular or membrane ef[illegible] have resulted in the high toxicity observed at 5.13 g/ℓ.

Results [illegible] Callen [illegible] study are presented in Table 10-1. A 1-hour treatment [illegible] CCl$_4$ at the highest amount tested (5.13 g/ℓ) resu[illegible] increases in gene conversion (31-fold) and mitotic cross[illegible] reversion was also increased, but to a lesser extent (3-fold increase). Survival was only 10% at this concentration of CCl$_4$. At the intermediate level (4.31 g/ℓ) of CCl$_4$, much smaller effects in the three genetic systems were observed (2- to 3-fold increases). Survival at this level was 77%. Thus, the data in the Callen et al. (1980) study are suggestive of a weak positive response, but additional studies are needed before it can be conclusively stated that CCl$_4$ causes genetic effects in yeast.

Negative results have been obtained in a recently developed in vitro chromosome assay that utilized an epithelial-type cell line derived from rat liver (Dean and Hodson-Walker, 1979). This cell line has sufficient metabolizing activity to activate various chemical mutagens and carcinogens without the need for an exogenous activating system. Sealed-flask cultures were treated for 22 hours with CCl$_4$ dissolved in growth medium at 0.005, 0.010 and 0.020 mg/ℓ. Carbon tetrachloride at these low concentrations did not induce any chromatid or chromosomal aberrations, whereas a number of direct-acting mutagens and several requiring metabolic activation produced chromatid deletions, gaps and exchanges. However, none of the other substances tested was a heavily chlorinated compound. In addition, one of these substances was assayed at levels comparable to that used for CCl$_4$ and the remaining 10 compounds were assayed at doses several orders of

TABLE 10-1

Genetic Effects of Carbon Tetrachloride on Strain D7 of *Saccharomyces cerevisiae* following 1-hour treatment at 37°C[a,b]

	Concentration (g/ℓ)			
	0	3.23	4.31	5.13
Survival				
Total colonies	1454	1252	1120	152
% of control	100	86	77	10
trp5 locus (gene conversion)				
Total convertants	285	331	350	506
Convertants/10^5 survivors	2.0	2.6	3.1	61.7
ade2 locus (mitotic crossing over)				
Total twin spots	1	3	3	10
Mitotic cross-overs/10^4 survivors	1.6	5.3	5.8	40.1
Total genetically altered colonies	11	19	16	65
Total genetically altered colonies/ 10^3 survivors	1.7	3.4	3.1	33.3
ilv1 locus (gene reversion)				
Total revertants	38	41	57	11
Revertants/10^6 survivors	2.6	3.3	5.1	7.2

[a] Source: Adapted from Callen et al., 1980

[b] The total number of colonies in the different classes represent total counts of colonies from five plates in the case of survival, conversion and revertant-frequency estimations. Mitotic crossing over was estimated from counts of colonies growing on a total of 30 plates, 20 plates containing medium on which all surviving cells grew and 10 plates containing medium on which only *trp5* convertants grew.

magnitude larger. The doses chosen for each substance assayed were determined from cytotoxicity assays. Apparently, CCl_4 was very toxic to rat liver cells. EDTA (0.1 mM) has been found to decrease the cytotoxicity of CCl_4 without affecting mutagenicity in bacteria (Cooper and Witmer, 1982), apparently by decreasing membrane lipid peroxidation (Masuda and Murano, 1977). Perhaps addition of EDTA to a mammalian in vitro chromosome assay, such as in the study carried out by Dean and Hodson-Walker (1979), would allow for the use of larger concentrations of CCl_4.

10.4. OTHER STUDIES INDICATIVE OF DNA DAMAGE

Mirsalis and Butterworth (1980) measured unscheduled DNA synthesis (UDS) in primary rat hepatocyte cultures following in vivo treatment of adult male Fischer-344 rats (200-250 g) with CCl_4 (certified ACS grade, Fischer Scientific Co.) by oral gavage. Control rats received corn oil by gavage. Acetylaminofluorine and dimethylnitrosamine were also tested. At 2 hours after treatment, the livers were perfused in situ and hepatocytes were isolated. Approximately 6×10^5 viable cells were seeded in culture dishes containing coverslips and allowed to attach to the coverslips for 90 minutes. After the coverslip cultures were washed, they were incubated in a medium containing 10 μCi [^{3}H]thymidine (42 Ci/mmol) per mℓ for 4 hours. The cultures were washed again and incubated in medium containing 0.5 mM cold thymidine for 14-16 hours. The extent of UDS was assessed by autoradiography. Net grains/nucleus were calculated as the silver grains over the nucleus minus the highest grain count of three adjacent nuclear-sized areas over the cytoplasm. The area of the silver grains was counted rather than the grain number, so that densely labeled cells where silver grains were touching could be accurately measured.

Cells from control animals ranged from -2 to -6 net grains/nucleus. Treatment of rats with dimethylnitrosamine (i.p.) at 10, 1 or 0.1 mg/kg yielded 36.6, 6.4 and -0.9 net grains/nucleus, respectively; dimethylnitrosamine at 10 mg/kg (p.o.) produced 22.2 net grains/nucleus. Oral doses of acetylaminofluorine at 50 or 5 mg/kg yielded 14.0 and 6.4 net grains/nucleus, respectively. Carbon tetrachloride at 100 or 10 mg/kg (p.o.) yielded -3.2 and -5.1 net grains/nucleus, respectively. Thus, dose-related increases in UDS were observed for dimethylnitrosamine and acetylaminofluorine, whereas CCl_4 produced no response.

In this study two doses of CCl_4 were tested: 10 mg/kg and 100 mg/kg. The oral LD_{50} for CCl_4 in rats is 2800 mg/kg. It [illegible]s not determined in this study at what dose hepatic cell toxicity would occur under the conditions used. Therefore, it is not clear that adequate doses of CCl_4 were tested. Also, it is not clear whether the 2-hour time period between exposure of the rats to CCl_4 and isolation [illegible] the hepatocytes was sufficient for observation of UDS. In a study by Popp et al. (1978), in which CCl_4-induced hepatocellular changes were noted, the shortest exposure period studied was 6 hours. If Mirsalis and Butterworth had shown that the 2-hour exposure period was sufficient for activation of the CCl_4 to a reactive intermediate, perhaps by demonstrating alkylation of protein by $^{14}\cdot CCl_3$, the negative results they obtained would be more convincing. Thus, this negative result could truly reflect the inability of CCl_4 to cause UDS in the system used or it could be a false negative result due to factors such as an inadequate dose or an inadequate exposure time period.

Craddock and Henderson (1978) carried out an _in vivo_ UDS study in which hepatocyte nuclei were isolated and then assayed for radioactivity by scintillation counting rather than by grain counting. This method may be superior to the whole-cell grain counting procedure used by Mirsalis and Butterworth (1980) as described above, particularly for chemicals that may cause low UDS activity, because a small effect could be obscured when background cytoplasmic grain counts are subtracted from the nuclear grain counts. In this study, Craddock and Henderson used a CCl_4 dose of 4000 mg/kg, which is significantly larger than the oral LD_{50} (2800 mg/kg). Negative results were obtained after a 2-hour exposure. A positive effect was observed after a 17-hour exposure. The authors suggested that this result may have been due to secondary effects such as lysosomal damage, which may result in release of DNA degradative enzymes.

In their latest study, Mirsalis et al. (1982) used combinations of doses (up to 400 mg/kg) and exposure times (up to 48 hours) that resulted in liver toxicity, as measured by an increase in liver cell mitosis. Therefore, the above mentioned criticisms of the previous studies relating to inadequate dose and exposure appear to have been obviated by this study. Carbon tetrachloride tested negative in this study also. However, it is still uncertain whether the method of assaying for UDS (subtraction of cytoplasmic grain counts from nuclear grain counts) will allow for detection of a weak response (see above). In addition, benzo(a)pyrene, 7,12-dimethylbenz(a)-anthracene and N-methyl-N'-nitro-N-nitrosoguanidine were negative in this in vivo UDS assay, whereas these chemicals tested positive in the in vitro rat hepatocyte UDS assay (Williams, 1981). This discrepancy suggests that the in vitro test may be more sensitive than the in vivo assay. Carbon tetrachloride has not been tested in the in vitro rat hepatocyte UDS assay.

In summary, the above in vivo UDS results suggest that CCl_4 may not damage DNA. However, in vitro UDS studies are needed before a decision on the DNA damaging potential of CCl_4 can be made. Also, use of a procedure for the UDS assay in which the radioactivity of isolated nuclei is assayed, rather than one in which grain counts of nuclei are corrected for grain counts in the cytoplasm, may better allow for detection of low levels of UDS.

10.5. SUGGESTED ADDITIONAL TESTING

Suggested additional testing falls into six categories:

1. The DNA damage studies reported by Craddock and Henderson (1978), Mirsalis and Butterworth (1980) and Mirsalis et al. (1982), suggesting that CCl_4 does not damage DNA following *in vivo* treatment of rats, should be corroborated by the *in vitro* rat hepatocyte UDS assay of Williams (1981). The *in vitro* test may be more sensitive than the *in vivo* test for potential weak genotoxic effects. Also, use of a procedure in which nuclei are isolated and assayed for radiolabel is recommended rather than use of the standard procedure in which cytoplasmic grain counts are subtracted from nuclear grain counts. The former procedure may better allow for detection of a weak response.
2. Studies using experimental procedures that are sensitive enough to detect the formation of liver DNA-chemical adducts after exposure of animals to CCl_4 are needed to confirm the low level of DNA binding observed by Diaz Gomez and Castro (1980a) and by Rocchi et al. (1973).
3. Additional data are needed to corroborate the Callen et al. (1980) study in the yeast system, which utilizes an endogenous activation system and is capable of assaying for point mutations, mitotic crossing over, and gene conversion. The Callen et al. study is the only yeast study reported in the literature.

4. Additional cytogenetic testing for chromosome effects in mammalian systems is needed before CCl_4 can be considered to be adequately tested for chromosome damage. Because EDTA has been reported to decrease cytotoxicity due to CCl_4 in bacteria (Cooper and Witmer, 1982), perhaps *in vitro* mammalian liver cell cytogenetic assays should be carried out in the presence of EDTA so that higher levels of CCl_4 could be assayed than were used in the Dean and Hodson-Walker (1979) study. In addition, *in vivo* cytogenetic studies such as the bone marrow micronucleus test, are needed.
5. Corroboration of the preliminary evidence for the weakly mutagenic response in the Ames test reported by Cooper and Witmer (1982) is needed. The same experimental conditions (fresh rabbit liver S9 and exposure of the bacteria in suspension to CCl_4 under reduced oxygen tension in the presence of EDTA) should be used and several concentrations of CCl_4 should be tested to establish whether or not a dose-response relationship exists. In addition, *in vitro* mammalian mutagenicity studies should be carried out using these same experimental conditions.
6. Studies on the ability of CCl_4 to reach reproductive organs and cause germ cell mutation were not available. If results from the tests suggested above for somatic cell mutation are positive, then studies assessing heritable risk are needed. (See Federal Register, 1980c for guidance on such tests.)

10.6. SUMMARY AND CONCLUSIONS

Carbon tetrachloride has been tested for its mutagenic potential in bacteria, yeast and a mammalian cell line and for its DNA damaging potential in rat hepatocytes when administered *in vivo*.

Six of the seven point mutation studies in bacteria were negative. The remaining bacterial study (Cooper and Witmer, 1982) was preliminary and only suggestive of a weak mutagenic response. In none of the negative studies was it shown that CCl_4 was activated or metabolized by the exogenous S9 activation system used. Metabolism of 2-aminoanthracene or vinyl compounds (used as positive controls) is probably an inadequate indication that the activation system can metabolize halogenated alkanes such as CCl_4. These substances are very different from CCl_4. A better indication that the activation system is sufficient for metabolism of CCl_4 may be to show that it metabolizes $[^{14}C]CCl_4$ to intermediates that bind to macromolecules. It is also conceivable that potentially mutagenic reactive intermediates of CCl_4 (such as the free radical $\cdot CCl_3$ and phosgene) are generated in the presence of an S9 activation system but that they are too short-lived to interact with DNA in _in vitro_ test systems.

The Callen et al. (1980) study was designed to overcome this problem by the use of an _in vivo_ activation system in yeast. Positive mutagenicity and DNA damage results were reported. In contrast, negative DNA damage results were reported, using _in vivo_ UDS as the assay endpoint. However, these negative results need confirmation by the potentially more sensitive _in vitro_ hepatocyte UDS assay of Williams (1981). Binding studies by Rocchi et al. (1973) and by Diaz Gomez and Castro (1980a,b, 1981) indicate that metabolically activated CCl_4 may interact with DNA.

Therefore, the negative genotoxicity test results that have been reported may be due to any of four (or more) factors: 1) CCl_4 is not mutagenic; 2) excessive volatilization and escape of CCl_4 if appropriate precautions are not taken; 3) inadequate activation of CCl_4 by the S9 system used to a metabolite capable of causing mutations (e.g., $\cdot CCl_3$ or phosgene), or

4) inability of any reactive intermediates formed to reach DNA before being scavenged by lipid and protein (particularly under conditions of exogenous activation, such as in the Ames test).

Additional tests should be conducted in which appropriate measures are taken to ensure that: 1) volatilization and escape of CCl_4 does not decrease exposure of the test organism or cell to levels of CCl_4 that are too low to be effective, 2) metabolic activation is occurring, and 3) DNA is exposed to the activated chemical species.

In summary, evidence described in this report is insufficient to allow firm conclusions to be made concerning the genotoxicity of CCl_4. The borderline results for binding of reactive intermediates to DNA (Rocchi et al., 1973; Diaz Gomez and Castro, 1980a, 1981), the study of Callen et al. (1980) and the highly preliminary evidence for mutagenicity in the Ames assay (Cooper and Witmer, 1982) are insufficient evidence for genotoxicity, but are suggestive of weak genotoxic effects and are an indication that further studies should be done.

11. CARCINOGENICITY

Carcinogenic effects of CCl_4 have been studied in rats, mice and hamsters. These studies are reviewed in Section 11.1. Human case reports and one occupational study are reviewed in Section 11.2.

11.1. EXPERIMENTAL ANIMALS

11.1.1. Rat Studies. Studies performed on rats have primarily used subcutaneous injection as the route of exposure. Hepatomas were found as well as toxic effects such as cirrhosis, hyperplasia and cholangiofibrosis. Neoplasms also developed following exposure by oral ingestion.

Cameron and Karunaratne (1936) looked at CCl_4 cirrhosis in relation to liver regeneration in the rat. Albino rats (strain not given) weighing ~150 g each were administered subcutaneous injections of 0.1-0.25 mℓ CCl_4 twice a week. Changes developed in the liver after 6-10 doses and disappeared within 7-10 days after cessation of treatment. With longer periods of exposure, the liver showed less and less tendency to return to a normal appearance when administration of the chemical was discontinued. Cirrhosis of the liver developed after several doses and was severe and irreversible after 40 doses.

Upon examination the liver was found to be pale, tough and finely granular. Extensive fibrosis radiated from the portal areas, thereby dividing the liver into small irregular masses. Hyperplastic nodules were seen in different parts of the liver.

In summary, rats given subcutaneous injections of CCl_4 readily developed cirrhosis of the liver. Hyperplastic nodules of the liver were also noted.

Reuber and Glover (1967a) administered subcutaneous injections of CCl_4 to inbred Buffalo male and female rats 4, 12, 24 and 52 weeks old. Additionally, newborn rats were obtained and given injections of CCl_4 at 4 days of age. Each age group contained 10-14 rats of each sex. Control groups consisted of six animals per each age and sex. All experimental animals were given 1.3 mℓ/kg bw of a 50% solution of CCl_4 in corn oil twice a week for 12 weeks. Control animals were injected with the same amount of corn oil.

The 4-day-old animals died in an average of 8 days with hepatic and renal necrosis. The other age-groups survived for the duration of the study. During this period, the 52-week-old rats maintained their weight, and the 12-week-old rats each gained from 20-30 g. The 4-week-old females weighed 3 times their starting weights and males weighed 4 times their starting weights.

At sacrifice complete necropsies were done. All organs were examined histologically, including such tissues as diaphragm, tongue and skeletal muscle. Special staining was done for glycogen, mucin, connective tissue, ceroid, canaliculi, hemosiderin and lipid.

The males given subcutaneous injections at 52 weeks of age had more hyperplastic lesions than the other males. Hyperplastic nodules were found in 6 of 14 (43%) rats, with one having a small hepatic carcinoma. The only other males with nodules were the 24-week-old rats, 2/11 (18%). The remaining 52-week-old and 24-week-old male rats had hyperplasia of the liver. Hyperplasia developed in less than half of the 12-week-old male rats. Hyperplastic lesions and hyperplasia were not observed in control male rats.

The 24- and 52-week-old females had more hyperplastic nodules than did the younger females. The most striking lesions were in the 24-week-old female rats. In this group, 8/10 rats (80%) had hyperplastic nodules and one rat had a small carcinoma of the liver. There were more hyperplastic nodules per liver and larger lesions in the females than in the males. Lesions were not present in control female rats.

There were two kinds of hyperplastic lesions in the liver, one located in the periportal region and the other around central veins. Cirrhosis varied from mild to severe, but was unrelated to the hyperplastic lesions in individual rats. The severity and the histologic pattern of the cirrhosis were related to age and sex. The hyperplastic nodules seen were similar to those known to be preneoplastic (Reuber and Glover, 1967a). If the study had been continued for a longer period of time, it is possible that the hyperplastic nodules could have become overt tumors. Results of this study are given in Table 11-1.

In summary, 24- and 52-week-old inbred Buffalo rats of both sexes given subcutaneous CCl_4 developed more hyperplastic hepatic nodules, as well as an occasional early carcinoma of the liver, than did rats of other ages. The number of hyperplastic lesions per liver and the size of the lesions were larger in females than in males. Four-day-old rats died with necrosis of the liver and kidney.

Reuber and Glover (1967b) also studied cholangiofibrosis of the liver in male and female Buffalo strain rats of varying ages. Rats were given subcutaneous injections of 1.3 mℓ/kg bw of a 50% solution of CCl_4 and corn oil twice weekly for 12 weeks. Cholangiofibrosis may be a precursor of cholangiocarcinomas of the liver (Reuber and Glover, 1967b); it is a lesion composed of ducts lined by irregular epithelial cells and surrounded by

TABLE 11-1

Lesions of the Liver in Rats Given Subcutaneous Carbon Tetrachloride* (1.3 mℓ/kg bw in 50% solution with corn oil)

Age (weeks)	Hyperplasia	Hyperplastic Nodules	Carcinoma	Total Nodules Plus Carcinoma
MALE				
4	6/14 (43%)	0/14 (0%)	0/14 (0%)	0/14 (0%)
12	4/11 (36%)	0/11 (0%)	0/11 (0%)	0/11 (0%)
24	8/11 (73%)	2/11 (18%)	0/11 (0%)	2/11 (18%)
52	7/14 (50%)	6/14 (43%)	1/14 (7%)	7/14 (50%)
FEMALE				
4	4/11 (36%)	0/11 (0%)	0/11 (0%)	0/11 (0%)
12	5/11 (45%)	3/11 (27%)	0/11 (0%)	3/11 (27%)
24	1/10 (10%)	8/10 (80%)	1/10 (10%)	9/10 (90%)
52	4/11 (36%)	6/11 (54%)	1/11 (9%)	7/11 (64%)

*Source: Reuber and Glover, 1967a

connective tissue. Cholangiofibrosis of the liver developed in male and female rats receiving injections of CCl_4. The lesion was present in male rats of all ages, except those 4 weeks of age. Some rats were fed 3-methylcholanthrene (MCA) in the diet in addition to receiving the subcutaneous CCl_4 injections. The lesion was increased in male rats 5 weeks of age given both CCl_4 and MCA, whereas it was decreased in rats of all other ages. Most female rats given both chemicals also had cholangiofibrosis.

The comparative carcinogenicity of CCl_4 has been studied in five rat strains: Japanese, Osborne-Mendel, Wistar, Black and Sprague-Dawley (Reuber and Glover, 1970). Groups of 12-17 male rats of each strain were given twice weekly subcutaneous injections of CCl_4 (2080 mg/kg bw as a 50% solution in corn oil) for varying durations due to the different survival patterns of these strains. Treated animals were killed when moribund; controls for each strain were killed at the same time as the last experimental animal. Incidence of hepatic lesions is given in Table 11-2. Lesions other than hepatic also occurred. Hemangiomas of the spleen were present in two Japanese rats and in one of the Osborne-Mendel strain. There were carcinomas of the thyroid gland in three Osborne-Mendel and three Japanese rats. One Japanese rat had a subcutaneous leiomyosarcoma; two Osborne-Mendel and three Japanese rats had chronic renal disease.

The data indicate that: (1) sensitivity to CCl_4-induced neoplasms varies widely among these five rat strains; and (2) the trends in incidence of neoplasms and severe cirrhosis appear to be inversely related. Varying amounts of toxicity occurred: all experimental animals of the Black rat strain were dead at 18 weeks, and those of the Sprague-Dawley strain at 16 weeks; the failure to find carcinomas in those strains may have been caused

TABLE 11-2

Incidence of the Most Advanced Hepatic Lesions in Rats Administered Carbon Tetrachloride[a] (2080 mg/kg bw in 50% solution with corn oil)

Lesion	Japanese	Osborne-Mendel	Wistar	Black	Sprague-Dawley
No hyperplasia	0/15	0/13	0/12	4/17	8/16
Hyperplasia	0/15	1/13	1/12	6/17	6/16
Hyperplastic nodule	3/15	4/13	7/12	7/17	2/16
Small carcinoma	4/15	4/13	3/12	0/17	0/16
Large carcinoma	8/15	4/13	1/12	0/17	0/16
Total carcinoma[b]	12/15	8/13	4/12	0/17	0/16
No cirrhosis	0/15	0/13	0/12	0/17	0/16
Mild cirrhosis	9/15	2/13	0/12	0/17	0/16
Moderate cirrhosis	5/15	7/13	6/12	4/17	0/16
Severe cirrhosis	1/15	4/13	6/12	13/17	16/16

[a] Source: Adapted from Reuber and Glover, 1970

[b] These incidence rates may not be comparable due to large differences in average survival for the different strains.

in part by an insufficient study duration. In all three other strains, toxicity (e.g., cirrhosis, hepatic vein thrombosis, cholangiofibrosis) occurred. Toxicity was inversely related to carcinogenicity. Development of liver carcinomas was related to the severity of cirrhosis and survival time. Sprague-Dawley, Black and to a lesser extent Wistar males died from moderate or severe cirrhosis before they could develop carcinomas. Japanese and Osborne-Mendel rats were less susceptible to cirrhosis; thus, they survived and developed carcinomas.

In the NCI bioassays for chloroform (NCI, 1976a), 1,1,1-trichloroethane (NCI, 1977) and trichloroethylene (NCI, 1976b), CCl_4 was used as the positive control. The positive control groups of 50 Osborne-Mendel rats of each sex were administered CCl_4 in corn oil by gavage 5 times weekly for 78 weeks at two dose levels: 47 and 94 mg/kg bw for males, 80 and 159 mg/kg bw for females. This treatment resulted in some toxicity (cirrhosis, fatty liver) and death. At 110 weeks using the high dose, only 7/50 males and 14/50 females survived; at the low dose, 14/50 males and 20/50 females survived as compared to 26/100 males and 51/100 females for controls. The median survival times were 92 and 102.5 weeks for males given the low and high doses, respectively, and 67.5 and 102.5 for females given the low and high doses, respectively. The incidence of hepatocellular carcinomas was increased in animals exposed to CCl_4 as compared to pooled colony controls (Table 11-3). However, this was statistically significant only for low dose females as compared to the colony controls and not the matched controls. Absolute incidence of hepatic neoplasms was low (~5% in the animals exposed to CCl_4). The apparent decrease in the incidence of hepatocellular carcinomas in female rats at the high dose was attributed to increased lethality (i.e., females died before tumors could be expressed). The incidence of

TABLE 11-3

Incidence of Liver Tumors in Carbon Tetrachloride-Treated Rats and Pooled Colony Controls*

Animal Group		Hepatocellular Carcinoma	Neoplastic Nodule
Males	Controls (pooled)	1/99	0/99
	Low dose (47 mg/kg bw)	2/50	2/50
	High dose (94 mg/kg bw)	2/50	1/50
Females	Controls (pooled)	0/98	2/98
	Low dose (80 mg/kg bw)	4/49	2/49
	High dose (159 mg/kg bw)	1/49	3/49

*Source: NCI, 1976a,b, 1977

other neoplasms was acknowledged but not quantified. The National Research Council used these data in estimating carcinogenic risks for CCl_4 (NAS, 1978).

11.1.2. Mouse Studies. Several studies have been reported which indicate induction of liver tumors in various strains of mice treated with CCl_4 either by oral ingestion (which has been the primary route of exposure), subcutaneous injection or rectal administration. Signs of liver toxicity, such as necrosis and cirrhosis, have also been a frequent result of CCl_4 treatment in the carcinogenicity studies in mice.

In a study by Andervont (1958), groups of 30-77 female or male C3H mice were administered 6.46 mg CCl_4 by gavage once weekly for 2 weeks, followed by administration of 9.6 mg CCl_4 once weekly for 17 weeks (equivalent to 213 and 320 mg/kg bw). Pathogen-free and pathogen-carrying C3H mice were used. For males, no difference in the incidence of hepatomas was observed between the pathogen-free and pathogen-carrying groups; thus, they were combined. The average number of hepatomas per animal was 1.8 in treated animals (pathogen-free and pathogen-carrying combined) and 1.3 in controls. In females, a difference between the average number of hepatomas in pathogen-free mice (1.5) and pathogen-carrying mice (1.2) was observed. In control females, the average number was 1.0. In males, the tumor incidence was 79% in treated animals (pathogen-free and pathogen-carrying combined) and 49% in controls. In females, the tumor incidence in pathogen-free mice was 46% and in pathogen-carrying mice was 29%. In control females, the incidence was 3% (Table 11-4). The average number of hepatomas per female mouse in the pathogen-free, pathogen-carrying and control groups was 1.5, 1.2 and 1.0, respectively, indicating that both the incidence as well as the average number of tumors per animal increased in the order: control mice < treated pathogen-carrying mice < treated pathogen-free mice.

TABLE 11-4

Occurrence of Hepatomas in Strain C3H Mice Following Administration of Carbon Tetrachloride[a,b]

Sex[c]	Number of Mice	Carcinogen	Mice without hepatomas Mice with hepatomas Age in Months								Totals	Tumor Incidence (%)	Average Number of Hepatomas in Tumor-Bearing Mice
			8	9	10	11	12	13	14	15			
Males	77	Carbon tetrachloride	--	1/0	0/2	5/10	5/24	2/6	3/16	9/3	16/61	79	1.8
Females+	42	Carbon tetrachloride	3/0	3/0	6/1	6/2	9/7	2/1	1/1	--	30/12	29	1.2
Females-	37	Carbon tetrachloride	--	--	--	3/2	9/4	3/5	5/6	--	20/17	46	1.5
Males	45	None	--	--	--	--	2/0	--	--	21/22	23/22	49	1.3
Females-	30	None	--	--	--	--	2/0	--	2/0	25/1	29/1	3	1.0

[a] Source: Andervant, 1958

[b] Strain C3H males show hepatoma incidence between 12 and 55% when 15 months old; breeding females show incidences between 5 and 11% when over 15 months of age.

[c] A + sign denotes agent-carrying and a - sign agent-free females.

A study by Edwards (1941) also reported the induction of hepatomas in mice by exposure to CCl_4. The animals used were 207 male C3H mice, aged 3-6 months, and 133 male and female strain A mice, aged 2-3.5 months. They were given 0.1 mℓ of a 40% olive oil solution of CCl_4 (0.04 cc CCl_4) by stomach tube two or three times weekly for 8-16 weeks. Autopsy was performed up to 21 weeks after the last treatment.

Olive oil was administered by stomach tube in doses of 0.1 mℓ 2 or 3 times weekly to control male C3H and A mice from the same stock as those used in treated groups. In total, 23 strain C3H mice were given CCl_4 from 39-50 times and were killed and examined from 9-11 months of age. A high percentage of the treated animals developed hepatomas.

Of 143 C3H mice, varying from 6-10 months of age at autopsy, 126 (88.1%) showed hepatomas (Table 11-5). Similar tumors were present in all of the 54 strain A mice whose ages varied from 4.5-12 months (Table 11-6). It should be noted that the incidence of spontaneous hepatomas in both the C3H and A strains is markedly below that of the induced tumors in the treated mice. Autopsies performed on 17 C3H male mice 8.5-9 months of age and of the same stock as that used in the study failed to show any hepatic tumors.

Edwards et al. (1942) performed another study on mice. The mice used were inbred strain L (their incidence of spontaneous hepatomas is extremely low), 2.5-3.5 months or 3.5-7.5 months of age at the start of the experiment. The number of mice varied from 8-39 per group. Carbon tetrachloride of a high degree of purity was administered in olive oil by stomach tube usually three, but occasionally two, times weekly. Each treatment consisted of 0.1 cc of a 40% solution or 0.04 mℓ of CCl_4. Mice were given 46 administrations of CCl_4 over a 4-month period and were killed and necropsied 3-3.5 months after the last treatment. The mice varied from 8.5-14 months of age at necropsy. The liver was examined histologically.

TABLE 11-5

Incidence of Tumors in C3H Mice Ingesting Carbon Tetrachloride*

Group	Number of Mice Autopsied	Number of Mice with Hepatomas	Incidence of Hepatomas (%)
Controls	17	0	0
Controls w/Olive oil	23	1	4.3
Treated Animals (Olive oil and 0.04 mℓ CCl_4)	143	126	88.1

*Source: Edwards, 1941

TABLE 11-6

Incidence of Tumors in Strain A Mice Ingesting Carbon Tetrachloride*

Group	Number of Mice Autopsied	Number of Mice with Hepatomas	Incidence of Hepatomas (%)
Controls	200	1	0.5
Controls w/Olive oil (0.1 mℓ 2 or 3x weekly)	22	0	0
Treated Animals (0.01 mℓ of a 40% soln. of CCl_4 in Olive oil 2 or 3x weekly)	54	54	100.0

*Source: Edwards, 1941

Hepatomas developed in 34/73 mice (47%) given CCl_4. Hepatomas were observed in 7/15 younger male mice (47%), 21/39 older male mice (54%), 3/8 younger females (38%), and 3/11 older females (27%). Cirrhosis of the liver was not mentioned. The results are summarized in Table 11-7.

Historically, the incidence of spontaneous hepatomas in strain L mice is extremely low: 2/152 (1%) in untreated mice. One of 23 untreated virgin male mice (4%) and 0 of 28 females (0%), necropsied at 15 months of age, had tumors of the liver. Tumors were not present in 22 males and 28 females 18 months of age or in 27 female breeders 12-23 months of age. One of 24 male breeders (4%) had a tumor.

In summary, strain L male and female mice were highly susceptible to the induction of hepatomas by CCl_4, and male mice were slightly more susceptible than female mice.

Eschenbrenner and Miller (1943) studied the effects of size and spacing of multiple CCl_4 doses in the induction of hepatomas. Strain A mice were used because of their normal low incidence of hepatomas in untreated mice ($\leq$1%). Male and female mice were 2.5-3 months old at the beginning of the study.

Solutions of CCl_4 in olive oil were administered by stomach tube. All mice received 30 doses of the solution or olive oil alone. Five dilutions of CCl_4 were used: 32, 16, 8, 4 and 2% solutions. Mice received 0.005 mℓ of a solution per gram bw containing 16×10^{-4}, 8×10^{-4}, 4×10^{-4}, 2×10^{-4} or 1×10^{-4} mℓ, respectively, of CCl_4. Control mice received 0.005 mℓ olive oil/g bw. Central necrosis of the liver was produced by each of the CCl_4 doses.

TABLE 11-7

Tumors of the Liver in Male and Female Strain L Mice Receiving Carbon Tetrachloride by Stomach Tube[a]

(0.04 mℓ CCl_4 2 or 3x weekly)

Age (months)	Males	Females
2.5 - 3.5	7/15 (47%)	3/8 (38%)
3.5 - 7.5	21/39 (54%)	3/11 (27%)
2.5 - 7.5[b]	28/54 (52%)[c]	6/19 (32%)[c]

[a] Source: Edwards et al., 1942

[b] These values represent total number of tumors observed in mice in both age groups.

[c] Old control mice of this strain exhibit a very low incidence, as compared to CCl_4-treated mice. Hepatomas were present in 2/71 untreated males (3%) and 0/81 untreated females (0%).

The experimental and control groups were divided into five subgroups according to the interval between successive doses (1, 2, 3, 4 or 5 days) and the total period of treatment (29, 58, 87, 116 or 145 days). Equal numbers of male and female mice were used in each of the experimental and the five control groups. All mice were examined for the presence of hepatoma 150 days after the first dose. Some of them were killed at that time; others were subjected to laparotomy. If hepatomas were not present, laparotomies were performed at monthly intervals thereafter to determine if hepatomas eventually did appear. The gross diagnoses of hepatoma were confirmed by histological examinations.

In the lower dosage and shorter interval groups, hepatomas were few in number and small in size. With increases in dose and in intervals between successive doses, there was progressive increase in the number of small hepatomas and the size of hepatomas for a given mouse. There was no difference in the incidence of hepatomas between males and females.

The authors present the data in a matrix showing dose by interval. An adaptation of their matrix is given in Table 11-8. Excluding the values for the 1-day interval, the data were summed across intervals and sexes. A χ^2 test for trend among proportions was significant at $p<0.01$. In this study, the incidence of hepatomas roughly increased nonlinearly with the time span between onset and termination of CCl_4 exposure. All animals were examined for the presence of hepatomas 150 days after the first dose.

TABLE 11-8

Hepatomas in Male and Female Strain A Mice Given CCl_4 via Stomach Tube[a]

mℓ of CCl_4/g bw 30 doses	Interval Between Doses (days)					Total Excluding Interval 1
	1	2	3	4	5	
16×10^{-4}						
Male	0/6[b]	5/7	6/6	5/8	2/4	
Female	0/6	3/5	2/6	6/7	4/5	33/48
8×10^{-4}						
Male	0/6	3/6	5/6	5/8	3/4	
Female	0/6	2/6	3/6	6/7	5/5	32/48
4×10^{-4}						
Male	0/6	0/6	4/6	6/8	2/4	
Female	0/6	3/6	4/6	3/7	3/4	25/47
2×10^{-4}						
Male	1/6	0/6	4/6	5/5	2/4	
Female	0/6	1/6	2/6	7/10	1/5	22/48
1×10^{-4}						
Male	0/6	1/6	4/6	7/8	1/4	
Female	0/6	0/6	3/6	5/7	2/5	23/48
0						
Male	0/3	0/3	0/3	0/2	0/2	
Female	0/3	0/3	0/3	0/3	0/3	0/22

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[a] Source: Adapted from Eschenbrenner and Miller, 1943

[b] Number hepatomas/number mice

In another study, liver necrosis and hepatomas were noted after NCI strain A mice were treated with CCl_4 for 4 months (Eschenbrenner and Miller, 1946). Treatment with CCl_4 was started when the mice were 3 months of age and was terminated when they were 7 months old. Groups of five male or female mice were administered CCl_4 by gavage at 0, 1200, 2400, 4800 or 9600 mg/kg bw. Dose schedules were either 120 doses in 119 days (daily) or 30 doses in 116 days (4-day intervals). Animals were necropsied at 8 months (30 days after cessation of administration). All mice were given one additional dose of the solution 24 hours prior to necropsy. Note that mice in the two groups at each dose level were administered the same total amount of CCl_4 over the same period of time, but with a variation in the number of doses into which the total amount was divided and, therefore, in the size of each dose. The doses for mice in group one (120 doses in 119 days) were previously determined as being "necrotizing" and "non-necrotizing" and in group two (30 doses in 116 days) as "only necrotizing." Two control groups of mice received 0.02 or 0.005 mℓ olive oil/g bw/dose. The incidence of hepatomas and necrotic lesions is noted in Table 11-9.

At the three high group one doses on the (120 doses in 119 days) dose schedule, a 100% incidence of hepatomas was observed. At the highest dose, the mice had hepatomas of up to 1 cm in diameter. At the two intermediate doses the neoplasms were smaller in size. No hepatomas were observed in the 1200 mg/kg group. The incidence of hepatomas was decreased in the animals on the group two (30 doses in 116 days) dose schedule as compared to those on the group one (120 doses in 119 days) dose schedule. At the 1200 mg/kg dose, however, very small hepatomas were detected by microscopic examination in two males. Mice given olive oil did not develop tumors.

TABLE 11-9

Susceptibility of Strain A Mice to Liver Necrosis and the Incidence of Hepatomas 30 Days After 120 or 30 Doses of Carbon Tetrachloride[a,b]

		Carbon Tetrachloride Dose														
		9600 mg/kg			4800 mg/kg			2400 mg/kg			1200 mg/kg			0 (olive oil)		
Sex	Dose Conditions (doses/days)	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
F	120/119	+	0/5	5/5	+	0/5	5/5	±	0/5	5/5	-	0/5	0/5	-	0/5	0/5
M	120/119	+	0/4	4/4	+	0/5	5/5	±	0/5	5/5	-	0/5	0/5	-	0/5	0/5
F	30/116				+	2/4	2/4	+	2/5	3/5	+	0/5	0/5	-	0/5	0/5
M	30/116				+	4/4	3/4	+	3/5	4/5	+	0/5	0/5[c]	-	0/5	0/5

[a] Source: Adapted from Eschenbrenner and Miller, 1946

[b] Based on gross and microscopic examination

[c] Two animals showing no tumor on gross examination were found each to have a very small hepatoma on microscopic examination of a random section of the livers.

A = Probable initial liver necrosis (+ = presence, - = absence)
B = Observed final liver necrosis
C = Hepatoma incidence

The presence or absence of hepatomas and of hepatic necrosis was determined. The localization of necrosis after chronic administration of CCl_4 did not appear to follow a definite pattern, in contrast to the regular pattern of centrilobular necrosis seen after a single dose was administered to strain A mice.

In summary, mice receiving non-necrotizing doses of CCl_4 developed as many, if not more, tumors of the liver than mice given necrotizing doses despite the fact that equal amounts of CCl_4 were administered. Mice given 1200 mg/kg did not develop gross tumors; most mice receiving either 2400, 4800 or 9600 mg/kg bw did develop tumors.

NCI performed bioassays on chloroform (NCI, 1976a), 1,1,1-trichloroethane (NCI, 1977) and trichloroethylene (NCI, 1976b) in which CCl_4 was used as the positive control. Tests were done using $B6C3F_1$ male and female mice (35 days of age, 50 per group). Treatment by oral gavage 5 times per week occurred for 78 weeks. Surviving mice were sacrificed at 92 weeks from the start of the study. The doses of CCl_4 were 1250 or 2500 mg/kg bw for mice of both sexes. A necropsy was performed on all mice along with complete histological examinations.

Most male and female mice treated with CCl_4 were dead by 78 weeks (Table 11-10). Median survival times were 63 and 72 weeks for the males given the low and high doses, respectively, and 58.5 and 68.5 weeks for the females given the low and high doses, respectively. Hepatocellular carcinomas were found in practically all mice receiving CCl_4, including those dying before termination of the test (Table 11-11). The first carcinomas were observed in low dose female mice at 16 weeks, in high dose female mice at 19 weeks, in low dose males at 48 weeks, and in high dose males at 26 weeks, compared to 90 weeks for vehicle control females and 72 weeks for

TABLE 11-10

Survival of B6C3F1 Mice Treated with Carbon Tetrachloride[a] (CCl_4 administered by oral gavage)

Dose	Initial	78 Weeks	91-92 Weeks
MALE			
Control			
Matched	20	13 (65%)	7 (35%)
Pooled	77	53 (69%)	38 (49%)
Low Dose[b]	50	11 (22%)	0 (0%)
High Dose[c]	50	2 (4%)	0 (0%)
FEMALE			
Control			
Matched	20	18 (90%)	17 (85%)
Pooled	80	71 (89%)	65 (81%)
Low Dose[b]	50	10 (20%)	0 (0%)
High Dose[c]	50	4 (8%)	1 (2%)

[a] Source: NCI, 1976a,b, 1977

[b] 1250 mg/kg bw

[c] 2500 mg/kg bw

TABLE 11-11

Incidence of Hepatocellular Carcinomas in Mice Treated with Carbon Tetrachloride[a]

(CCl_4 administered by oral gavage)

Dose	Hepatocellular Carcinomas
MALE	
Pooled Controls	5/77 (6%)
Low Dose[b]	49/49 (100%)
High Dose[c]	47/48 (98%)
FEMALE	
Pooled Controls	1/80 (1%)
Low Dose[b]	40/40 (100%)
High Dose[c]	43/45 (96%)

[a] Source: NCI, 1976a,b, 1977

[b] 1250 mg/kg bw

[c] 2500 mg/kg bw

vehicle control males. Some liver toxicity occurred, identified as cirrhosis, bile duct proliferation, toxic hepatitis and fatty liver; however, these cases were few in number. In summary, this study found CCl_4 to be highly carcinogenic for liver in mice.

Confer and Stenger (1966) studied nodules in the livers of C3H mice after long-term CCl_4 administration. Twenty-five male mice, 5 weeks of age, received rectal instillation of 0.1 mℓ of a 40% solution of CCl_4 dissolved in olive oil 2 times a week for 20-26 weeks. Only olive oil was given to 10 control mice. Fourteen mice were killed 9 days after the last treatment, and the remaining mice were killed at periods of 3-37 weeks. The livers were examined by light and electron microscopy and revealed that 5/14 mice (36%) killed after 9 days and 8/11 mice (73%) killed later developed hyperplastic hepatic nodules. Cirrhosis was not observed in the liver.

In summary, mice given CCl_4 by rectal instillation had hyperplastic nodules that persisted after the discontinuation of the chemical, but did not develop cirrhosis of the liver. Confer and Stenger (1966) proposed that hyperplastic nodules, as observed in their study, are precursors of liver carcinomas.

Edwards and Dalton (1942) studied the CCl_4 induction of cirrhosis of the liver and hepatomas in mice. They investigated the outcome of high dose, low dose and limited treatment. For high dose administration, strain C3H male mice, male and female strain A mice, male and female strain Y mice and strain C female mice were used. All mice were started in the study at 1-5 months of age.

In one experiment, a dose of 0.1 mℓ of a 40% solution of CCl_4 (with no impurities) in olive oil was administered by stomach tube 2 or 3 times per week. The total number of treatments varied from 23-58. In order to study any early pathologic changes, a number of mice were killed after receiving 1-23 doses. In another experiment, male mice were given 0.1 mℓ of olive oil 2 or 3 times a week for 39-62 doses.

Animals were killed at 1 year of age or younger by cervical dislocation. Subcutaneous transplants of tumor tissue were made by the trocar technique into mice of homologous strains. Special histological techniques were used to examine a number of primary and transplanted tumors. These included techniques for the presence of fat, glycogen or alkaline phosphatase and those for studying the mitochondria and Golgi apparatus.

Hepatomas were observed in 88% of C3H male mice treated with CCl_4, whereas they occurred in 4% of untreated mice of the same age and strain. Liver tumors developed in 60% of male and female strain Y mice treated with CCl_4 and occurred in only 2% of untreated mice of that strain. Liver tumors were seen in 98% of strain A mice of both sexes, whereas only 2% of these mice developed the tumor spontaneously. Hepatic tumors were found in 83% of strain C females, compared with 0% of untreated mice of the same age and strain. The hepatic tumors observed in this study were usually multiple -- as many as 10 occurring in one liver. Results of both the treated and control groups are given in Tables 11-12 and 11-13. Tumors did not appear to have been induced in any of the other organs.

Low dose administration (0.1 mℓ of 5% CCl_4 in olive oil or 0.005 mℓ) occurred 3 times weekly by stomach tube to 58 strain A female mice, 2.5 months of age, for 2 months. Mice were necropsied 2 days to 4.5 months after the last treatment. Hepatomas were present in 41 mice (71%), and some had cirrhosis of the liver.

TABLE 11-12

Hepatomas in Male and Female Mice Given Carbon Tetrachloride (0.04 mℓ 2-3x weekly) by Stomach Tube*

Strain	Age (months)	Males	Females	Both
C3H	6-10	126/143 (88%)	-	-
Y	4-12	-	-	9/15 (60%)
C	6-7	-	34/41 (83%)	-
A	4-12	-	-	161/164 (98%)

Hepatomas in Untreated Male and Female Mice

Strain	Age (months)	Males	Females	Both
C3H	8-11	2/50 (4%)	-	-
C3H	12-19	86/320 (27%)	-	-
Y	10-16	-	-	3/129 (2%)
C	13-24	-	0/150 (0%)	-
A	4-8	-	-	0/400 (0%)
A	12-16	-	-	8/400 (2%)

*Source: Edwards and Dalton, 1942

TABLE 11-13

Hepatomas in Male Mice Given Olive Oil by Stomach Tube*

Strain	Age (months)	Incidence
C3H	10-11	4%
C	12	0%
A	5-12	0%

*Source: Edwards and Dalton, 1942

The total dose (0.125-0.145 mℓ CCl_4) is comparable to the total dose of 0.120 mℓ CCl_4 in the experiment in which mice were given treatments of 0.04 mℓ each. The tumors of the liver were morphologically similar in both studies.

Limited treatment involved strain A female mice, 2 months of age. There were 21-62 mice in three treatment groups. The CCl_4 used was dissolved in olive oil, the volume of the mixture administered amounting to 0.1 mℓ. The mice were given 1-3 treatments. The hepatotoxic doses were 0.04, 0.01 or 0.005 mℓ CCl_4. Eleven mice received olive oil only. The mice were necropsied 2-12 months after the start of the experiment.

Tumors of the liver were not found in these mice. There was pigment in Kupffer cells, occasional foci of basophilic debris, and an increase in connective tissue and reticulum.

In summary, CCl_4 induced significant numbers of tumors of the liver, as well as cirrhosis, in four strains of mice.

Since successful transplantation is frequently considered to be a criterion of neoplasia, Leduc and Wilson (1959) attempted to transplant CCl_4-induced tumors of the liver in mice. At first

> "numerous failures to establish a transplantable CCl_4-induced hepatoma supported the idea that, if transplantability is a criterion, the nodules might be hyperplastic but not neoplastic. Subsequently, however, several such nodules were successfully transplanted from a host that was allowed to live for a long period after the CCl_4 administration ceased" (Leduc and Wilson, 1959).

Male mice of the BUB strain were used. Spontaneous hepatomas have not been found in this strain, up to its 40th generation. Carbon tetrachloride was administered by stomach tube in doses of 0.1 mℓ of a 40% solution in olive oil (0.04 mℓ CCl_4) per treatment. Carbon tetrachloride was given 3 times a week for a total of 45-66 doses. About one-third of the mice were given three daily intravenous (i.v.) injections of 0.2 mℓ of thorotrast before CCl_4 administration was started. As discussed by Leduc and Wilson (1959), thorotrast is useful in the detection of hepatomas but has been involved (as indicated by other investigators), in tumor production.

The first-generation tumor transplants were made subcutaneously. Subsequently, both subcutaneous and intrasplenic transplants were made. Under light ether anesthesia, implants of tumor into the spleen were made by an incision through the dorsal body wall. The spleens were examined periodically by laparotomy.

Hepatomas did not develop in 20 control mice given thorotrast only. Hepatomas did occur in CCl_4-treated mice that were free of thorotrast.

The CCl_4 hepatomas (5 of 7) that were successfully transplanted differed from those that did not grow in new hosts because a longer time period elapsed between CCl_4 administration and tumor transplantation. The five successful transplants were obtained from a single host killed 8 months after the last treatment, whereas those that did not grow were transplanted ~11 weeks after the last treatment. The authors concluded that chronic CCl_4 injury to the liver induces the development of both hyperplastic nodules and hepatomas. They found the livers of CCl_4-treated mice to be cirrhotic with numerous hyperplastic nodules.

11.1.3. Hamster Studies Hamsters have not been studied as extensively as rats and mice. There has been only one report of the induction of tumors in hamsters by CCl_4.

Della Porta et al. (1961) orally administered CCl_4 to Syrian golden hamsters as a part of a larger investigation of the response of this species to carcinogens that induced liver neoplasms in other species. Ten female and 10 male Syrian golden hamsters, 12 weeks old, were used. At the beginning of the experiment, males weighed an average of 99 g and females weighed an average of 109 g. At the end of the experiment, the average weight was 104 g for both sexes. The treatment consisted of weekly administration by stomach tube of a 5% solution of CCl_4 in corn oil for 30 weeks. Controls cited were historical controls kept by the investigators in the same laboratory for a lifespan. A total of 145 female and 109 male hamsters of the same strain, fed the same diet, did not develop liver-cell tumors. The authors also cited controls for a different study they conducted. In this latter study, 30 female and 50 male hamsters fed the same diet but given 0.5 mℓ corn oil via stomach tube twice weekly for 45 weeks also did not develop liver-cell tumors. During the first 7 weeks of the former experiment, 0.25 mℓ of the solution containing 12.5 μℓ CCl_4 was given each week. This dose was then reduced to 0.125 mℓ and contained 6.25 μℓ of CCl_4. After this treatment, the survivors were kept under observation for 25 additional weeks and then killed. Detailed histopathological examinations of all hamsters were conducted, except for one female lost through cannabalism at the 28th week.

Weights of the hamsters varied irregularly during the period following treatment. In general, the weights increased. Females weighed an average of 114 g and males 118 g. One female died at the 10th week of treatment; three females and five males died or were killed between the 17th and the 28th week. Three females died at weeks 41, 43 and 54. The surviving three females and five males were killed at the end of the 55th week.

Hamsters dying during the treatment and at the 41st week had cirrhosis, as well as hyperplastic nodules that were two to several layers thick. The cells showed irregularities in the shape, size and staining qualities of their cytoplasm and nucleus, with an uneven distribution of glycogen.

All of the animals, five males and five females, dying or killed 13-25 weeks after the end of the treatment, had one or more liver-cell carcinomas (a total of 22 tumors: 12 in the females and 10 in the males). No mention was made of toxicity in these animals. Liver cell carcinomas were not found in the other animals dying before week 43.

Transplantation efforts were not successful. The authors note in their discussion that this negative result deserves further investigation since "many other tumors of hamsters have been successfully transplanted to non-inbred hamsters in this and other laboratories."

In summary, Syrian golden hamsters appear sensitive to the carcinogenic effects of CCl_4. Although the number of animals in this study was small, the authors considered the results to be significant because the reported historical control incidence of hepatic tumors in hamsters was 0/254. Hyperplastic nodules appeared during treatment, and carcinomas appeared after CCl_4 administration had been discontinued, which suggests that the nodules or benign tumors were precursor lesions for carcinomas. It should be noted that this study is the only report found in the available literature of CCl_4 induction of tumors in hamsters.

It is recognized that differences exist among species regarding their sensitivities. However, data quantifying such differences have not been found in the available literature. Nevertheless, such differences should be noted in light of the predominance of research/data on the rodent species. Methods of dose conversion from animals to humans are discussed in the Appendix.

In concluding this section on animals, it should be noted that some of the research reported suggests that hepatomas occur only after liver necrosis and fibrosis have occurred (Edwards, 1941; Edwards and Dalton, 1942;

Della Porta et al., 1961; Reuber and Glover, 1967, 1970). The results have been interpreted to mean that "as far as the liver is concerned, hepatoma is an occasional consequence of the induction of postnecrotic cirrhosis and that CCl_4 is not a direct liver carcinogen" (Louria, 1977). The results reported by Eschenbrenner and Miller (1946), however, refute Louria's statement. These authors concluded that if CCl_4 is, in fact, a carcinogenic agent, tumors should be obtained with non-necrotizing doses. As discussed earlier in this chapter, their series of experiments examining the issue, revealed:

> "While it was found that a correlation exists between the degree of liver necrosis and the incidence of hepatomas in relation to dose, the use of a graded series of necrotizing and non-necrotizing doses indicated that repeated liver necrosis and its associated chronic regenerative state are probably not necessary for the induction of tumors with carbon tetrachloride" (Eschenbrenner and Miller, 1946).

The small number of animals used in this study must be noted.

A list of authors addressing the issue of liver necrosis induced by CCl_4 is provided in Table 11-14.

11.2. HUMANS

11.2.1. Case Reports. As mentioned in the section dealing with human toxicity, in many of the case reports the investigators present the data in narrative form. Although interesting, these type of data are not suitable to quantitative analysis since numbers are not adequately presented. Furthermore, there usually are a number of uncontrolled variables (alcohol intake, age, simultaneous exposures) or unknown variables (exposure amount) making it difficult to attribute the outcome solely to the CCl_4 exposure. Despite these limitations, they are important since they can lend support or challenge the experimental animal studies and can aid qualitatively in the extrapolations from animals to humans.

TABLE 11-14

Studies in Which Liver Necrosis was Induced Using Carbon Tetrachloride

Species	Route	Reference
Mice	gavage	Edwards, 1941
Mice	ingestion	Edwards and Dalton, 1942
Mice	ingestion	Eschenbrenner and Miller, 1946
Hamsters	ingestion	Della Porta et al., 1961
Rats	subcutaneous injection	Reuber and Glover, 1967a
Human	ingestion	Hashimoto et al., 1968
Rats	subcutaneous injection	Reuber and Glover, 1970

The carcinogenic effect of exposure to CCl_4 in humans has been suggested in a number of case reports by physicians. One of these was reported by Tracey and Sherlock (1968). A 59-year-old man with a history of moderate alcohol consumption returned from a cocktail party and noticed the vapor of CCl_4 used to clean a rug in his apartment earlier that evening. Five days later he developed nausea, vomiting and diarrhea and within 10 days of exposure he developed jaundice. The patient recovered following a long and complex hospitalization and was discharged after 9 weeks. Four years after hospitalization for jaundice, he was found to have a smooth, enlarged, nontender liver. He denied alcohol consumption within the intervening period. Three years after this checkup, the patient was readmitted with a history of nausea, vomiting and diarrhea. He again denied ingestion of alcohol. A liver biopsy was diagnosed as hepatocellular carcinoma. Postmortem examination revealed the liver to be extensively involved with tumor. Little normal liver tissue remained.

Aside from the acute exposure to CCl_4 7 years before diagnosis of cancer, the patient's possible additional exposure to this and other toxic chemicals was not reported. No medical history was given for the 3 years before final diagnosis.

Other case reports of human neoplasms developing after exposure to CCl_4 have appeared. In one, a woman developed modular cirrhosis of the liver followed by cancer of the liver after exposure to CCl_4, and died 3 years after the first exposure (Johnstone, 1948). However, she had suffered from periodic jaundice for 5 years prior to the CCl_4 exposure. In a second, a fireman developed cirrhosis and an epithelioma of the liver 4 years after acute CCl_4 intoxication (Simler et al., 1964). In none of the cases could a causal link between CCl_4 exposure and development of neoplasms be established.

11.2.2. Studies. A study by Capurro (1979) reports a series of cancer cases in a rural valley polluted by vapors from a solvent recovery plant. Odor and pollution problems existed in the valley from 1961-1971. A variety of solvents including CCl_4 were identified in the air. Blood tests were done on 24 residents; solvents were detected in the blood of all those tested. A list of these solvents detected is not given but those "most easily detected" (as cited by the author) were: benzene, chloroform, methyl isobutyl ketone and trichloroethylene. Levels were not reported.

A study population was defined consisting of 117 Caucasians who lived within 1.5 km^2 of the plant during the appropriate time for greatest exposure. These persons were followed for a 6-year period.

Ten cancer cases occurred during this time period including two cases that were not residents. The author's analysis focused on four cases of lymphoma (2 lymphosarcoma, ICD 200.0 and 2 reticulum cell sarcoma ICD 200.1). All four of these cases had a history of having worked in the paper mill which preceded the solvent recovery plant at the same location. The author reported that a study of paper mill workers (Milham, 1976) found that the proportionate mortality ratio [PMR = (observed deaths/expected deaths) x 100] increased 1.8 times for lymphosarcoma. No significant increase was found for reticulum cell sarcoma. However, as Capurro (1979) reported, "the excess lymphomas observed by us is far above the one described by Milham." A mortality ratio indicating a 160-fold increase for these two cancers (ICD 200.0 and 200.1) was calculated. However, it should not be reported due to errors in its calculation. Three observed deaths were used, rather than four, with no explanation and the study population was rounded off to 120 instead of using the defined 117. The ratio was not standardized by age or sex, nor was it described as race-specific (all Caucasians). The assumption was made that since the age distribution of the study population is comparable to that of the nation, age standardization was not necessary. However, the study population was then compared to the population of the State of Maryland rather than the nation. The discussion of lymphomas diagnosed in the county and in the area surrounding the plant lacks important information on population distribution, time period and comparison group as well as methods used. Due to its lack of specificity and its questionable statistical methods, this study is of limited value. Thus, in light of these deficiencies, the conclusion of the author that the incidence of malignant lymphoma is abnormal in the area exposed to solvent vapors is not supported.

In a preliminary study designed to determine if occupational exposure to CCl_4, trichloroethylene and tetrachloroethylene resulted in increased mortality, Blair et al. (1979) studied causes of death in 330 laundry and dry cleaning workers. Sex, race and age along with underlying and contributing causes of death were abstracted from death certificates. The underlying cause was classified according to the International Classification of Diseases, Seventh Revision, by a trained nosologist. The control standard was the age, race, sex and cause specific distribution of U.S. deaths from the same time period. The PMR for all malignant neoplasms was 128 (100 would signify no difference from the comparison group) and statistically significant ($x^2 = 6.423$, 1 df, significant at $p<0.05$). The excess deaths consisted of lung, cervical and liver cancers, and leukemia. The authors note that the lung and cervical cancer excess may reflect the lower socioeconomic status of these workers. The slight excess of liver cancer is consistent with bioassay studies showing liver abnormalities. The authors conclude that epidemiologic studies of this occupational group are warranted due to the increased risk observed.

The paper has numerous faults. First, the authors place too much confidence in the PMR. The PMR does not estimate risk but instead indicates the possibility of an increased risk. Any conclusions made by the authors concerning an increased risk associated with the exposure is unfounded. The exposed population is compared to the United States population to determine the expected number of deaths. Ideally, the exposed population should have been compared to another working population to control for the "healthy worker effect." However, more serious reservations arise when examining the data and conclusions. Based on the United States population, 126 deaths due to circulatory diseases were expected. One hundred deaths due to circulatory diseases were observed in the exposed population. This lower than expected number of deaths is, as noted by the authors, necessarily associated with the elevated PMR for cancer deaths (68 expected, 87 observed). Thus, it is difficult to conclude that the elevated PMR for cancer deaths is in excess of expectations but rather that it may merely be the result of lower than expected relative frequencies of other causes of death. Therefore, one can only conclude from this study that there is a need for additional work on this occupational group to clarify the issues raised. An excess risk of carcinogenicity following exposure to CCl_4 is not indicated.

Finally, increases in certain types of skin cancers can be attributed to increased atmospheric CCl_4. As explained in Chapters 5 and 6, CCl_4 may result in an overall reduction of stratospheric ozone, eventually leading to increased UV-B radiation. It is estimated that a 2 to 5% increase in basal cell skin cancer will occur per 1% decrease in ozone and that a 4 to 10% increase in squamous cell skin cancer will occur per 1% decrease in ozone (NAS, 1982). The relationship between sunlight and malignant melanoma is unclear, thus rendering quantitative estimates uncertain. However, sunlight may be a causal factor in the development of malignant melanoma.

11.3. SUMMARY

11.3.1. Experimental Animals. Carbon tetrachloride has been reported to be carcinogenic in numerous animal studies. Hepatocellular carcinomas have been the neoplasm induced in all species evaluated (rats, mice and hamsters). An increase in adrenal tumors in male and female mice was seen in one study. Hamsters, although only used in one study, have been the most sensitive species studied, followed by mice and then rats. A difference in sensitivity to CCl_4-induced neoplasms has been observed among five rat strains. Female rats have appeared less sensitive to the chronic toxic effects but more sensitive to the carcinogenic effects of CCl_4.

11.3.2. Humans. A number of cases of hepatomas appearing in humans years following exposure to CCl_4 have been reported. A study examining the effect of solvent vapors (one of which was CCl_4) on a group of environmentally exposed people concluded the existence of an abnormal incidence of malignant lymphoma. However, it should not be used as evidence of the carcinogenicity of CCl_4 due to the concomitant exposures and poor study techniques. A preliminary epidemiological study of a group occupationally exposed to CCl_4 revealed a slight excess of liver cancer. Although an excess risk of carcinogenicity following exposure to CCl_4 is not indicated, this study does indicate the need for additional research.

11.3.3. IARC Evaluation. The International Agency for Research on Cancer (IARC, 1979) has devised a rating scheme for carcinogenic potential based on animal and human studies. The IARC evaluations for CCl_4 are:

> "sufficient" evidence for carcinogenicity in animals, "inadequate" evidence for carcinogenicity in humans, an overall evaluation that CCl_4 is "probably carcinogenic to humans" (group 2B).

The animal category of "sufficient evidence" indicates that increased incidence of malignant tumors was shown in multiple species or strains, or in multiple studies, or to an unusual degree regarding incidence, site, tumor type, or age of onset.

The human category of "inadequate evidence" indicates that few data exist, the available positive evidence did not exclude chance, bias or confounding, and/or studies exist which did not show evidence of carcinogenicity.

The overall evaluation of "probably carcinogenic to humans", group 2B, reflects the important role played by the results of animal studies, with limited or no data on humans. The combination of "sufficient" evidence for animals and "inadequate" evidence for humans usually results in the overall classification of 2B.

11.3.4. Conclusion. In conclusion, there is evidence that CCl_4 may be a human carcinogen based upon the following studies: (1) positive findings on mice in the NCI bioassay for trichloroethylene (1976) in which CCl_4 was used as the positive control; (2) the hamster study by Della Porta et al., (1961); (3) the rat studies by Reuber and Glover (1967, 1970) and NCI (1976); and (4) the mice studies by Edwards (1941), Edwards et al. (1942), Edwards and Dalton (1942) and Weisburger (1977). Human data as reported by Blair et al. (1979) indicate the need for additional epidemiological research. The combined evidence of animal and human studies has been evaluated by the IARC as indicating that this compound is probably carcinogenic for humans.

12. SYNERGISM AND ANTAGONISM

12.1. SYNERGISM

A description of the entire clinical picture of CCl_4 toxicity should consider the role played by alcohol in the genesis of severe CCl_4 poisoning (von Oettingen, 1964). A number of researchers have reported on this phenomenon (Stevens and Forster, 1953; Kirkpatrick and Sutherland, 1956; Joron et al., 1957; New et al., 1962; Markham, 1967). It has been established that habitual ingestion of alcoholic beverages and also their occasional use may increase the dangers from comparatively moderate exposure (Markham, 1967; Tracey and Sherlock, 1968). This fact is illustrated by reports on simultaneous exposure of abstinent persons and consumers of alcohol to the same CCl_4 concentration with only the latter becoming seriously ill (von Oettingen, 1964).

An example of this was given by Smetana (1939) who reported on three cases of CCl_4 poisoning. In one case, a 35-year-old male dry cleaner/interior decorator (a "steady and heavy drinker") had been cleaning furniture and draperies with CCl_4. Several hours later, he developed the typical acute symptoms of dyspnea, cough and bloody sputum associated with exposure to CCl_4. His condition worsened and he subsequently died. A coworker of his, a teetotaler, had been working in the same room for the same amount of time receiving the same exposure to the CCl_4. The author stated that "although he felt the effect of the exposure and suffered from headache and gastrointestinal distress, he recovered quickly after breathing fresh air" (Smetana, 1939).

Hypotheses have been advanced to rationalize this apparent synergistic reaction between alcohol and CCl_4. Lamson et al. (1928) postulated that the alcohol either caused a greater absorption from the gastrointestinal tract or a greater penetration into the liver. Smetana (1939) theorized that the reduction of the glycogen store in the hepatic cells of alcoholics may have some role in the greater susceptibility to CCl_4 poisoning. This latter theory is supported by the findings that neonates are protected against CCl_4 poisoning and that neonates have an increased concentration of liver glycogen (Bhattacharyya, 1965).

Traiger and Plaa (1971) investigated the potentiating capacity of aliphatic alcohols on CCl_4 toxicity. Male Swiss-Webster mice were given a single dose by gavage of methanol, ethanol or isopropanol equivalent to 50% of the LD_{50}. After 20 hours, an i.p. injection of 0.0075 mℓ/kg CCl_4 in corn oil was administered. Controls received only the corn oil. Blood samples were analyzed for SGPT activity. All alcohols produced increased activity but the effect was most marked among the mice pretreated with isopropanol. The administration of the alcohols or CCl_4 alone did not change the SGPT levels.

In an effort to ascertain the differences of the alcohols in the potentiation of CCl_4, the authors performed more detailed biochemical studies on male Sprague-Dawley rats. In these experiments, a single dose of ethanol or isopropanol was administered by gavage 3-48 hours prior to the i.p. injection of 0.1 mℓ/kg CCl_4 in corn oil. Controls received only the corn oil. After 24 hours, the animals were sacrificed and blood and liver aliquots were taken for SGPT, glucose-6-phosphatase, bilirubin and hepatic triglyceride analysis. Isopropanol produced a more marked change in the activities of SGPT, glucose-6-phosphatase and triglycerides. Isopropanol pretreatment produced hyperbilirubinemia whereas ethanol pretreatment or CCl_4 alone at a 10-fold increase in dosage did not. Also, a 10-fold dosage increase in CCl_4 alone produced an increased level of SGPT activity; however, it was only ~25% of the SGPT activity found among rats pretreated with isopropanol.

Wei et al. (1971) investigated the potentiation of CCl_4 hepatotoxicity by ethanol and cold. Sprague-Dawley rats were exposed to either ethanol (50% v/v in water intubated per os), cold (2-4°C for 18 hours) or warmth (31-33°C for 18 hours). Indices of hepatotoxicity were SGPT levels and liver triglyceride levels. In both male and female rats, the SGPT levels increased after both ethanol and cold exposures in response to the CCl_4. The authors postulate that the ethanol as well as cold, stimulates release of norepinephrine, which in turn increases the susceptibility of the liver to CCl_4. Warmth was found to reduce the potentiating effects of ethanol on serum enzyme release.

According to Davis (1934), very obese or undernourished persons suffering from pulmonary diseases or gastric ulcers or having a tendency to vomiting, liver or kidney diseases, diabetes or glandular disturbances are especially sensitive to the toxic effect of CCl_4 (von Oettingen, 1964).

Strubelt et al. (1978) found that CCl_4-induced liver damage was significantly greater in rats concomitantly exposed to ethanol than in control rats not exposed to ethanol. Male Wistar rats were given either a 5% or 15% ethanol solution as their sole source of fluid (11.4 or 24.9% of total colonies, respectively). Controls were provided with tap water. Following 1, 2 and 3 weeks of exposure, CCl_4 was administered intraperitoneally at a dose of 0.1 mg/kg. Hepatotoxic effects were evaluated by measuring the serum activities of SGOT, SGPT and sorbital dehydrogenase (SDH) as well as histological investigations.

The potentiation noted was fully developed following 1 week of exposure and was greater in those rats provided a 15% ethanol solution than in the rats provided a 5% ethanol solution, thus appearing to be dose-dependent.

Hasumura et al. (1978) also examined the potentiation of CCl_4 hepatotoxicity by ethanol. They designed their study to determine if hepatic microsomal changes, which are secondary to chronic ethanol consumption, play a role in the mechanism of CCl_4 hepatotoxicity. Rats were pair-fed a liquid diet containing ethanol (36% of calories) or isocaloric carbohydrate for a period of 4-5 weeks. Carbon tetrachloride, at a dose of 0.5 mℓ/kg, was administered intragastrically 15 hours after ethanol withdrawal. Within 24 hours there was an increase in liver lipids and serum ornithine carbamyl transferase activity and a decrease in the activities of hepatic aminopyrene N-demethylase and glucose-6-phosphatase. These changes were determined to be significantly greater in the ethanol-fed rats versus the control rats indicating in vivo potentiation of CCl_4 hepatotoxicity by chronic ethanol consumption, even in the absence of ethanol at the time of exposure (Hasumura et al., 1978).

In an attempt to determine the mechanism of this effect, liver microsomes were incubated with $[^{14}C]CCl_4$ and a NADPH-generating system. The authors found an enhancement of: 1) the covalent binding of ^{14}C to microsomal protein in vitro, and 2) the biotransformation of $[^{14}C]CCl_4$ to $^{14}CO_2$ in vitro. This suggests that ethanol pretreatment stimulates the microsomal formation of an active metabolite of CCl_4. Thus, microsomal changes are responsible, at least partially for the increased CCl_4 hepatotoxicity (Hasumura et al., 1978).

In addition to ethanol, other compounds have been investigated as to their effect on CCl_4 hepatotoxicity. Curtis et al. (1979a) and Davis and Mehendale (1980) have studied the potentiation of CCl_4 hepatotoxicity by exposure to chlordecone (Kepone®).

Curtis et al. (1979a) found greatly potentiated hepatotoxicity reflected in the form of elevated SPGT and SGOT activities between groups of rats which had undergone a 15-day feeding of 0 or 10 mg/kg chlordecone, and a single intraperitoneal challenge of CCl_4. The CCl_4 challenge was either 0, 0.025, 0.05, 0.1 or 0.2 mℓ/kg. The SPGT and SGOT activities increased in excess of 30-fold and 10-fold respectively in chlordecone-fed rats challenged with CCl_4 of 0.1 and 0.2 mℓ/kg. The authors concluded that the data indicate a great potential for the production of severe liver damage resulting from interactions of CCl_4 and chlordecone exposure at levels which may be independently nontoxic.

Davis and Mehendale (1980) conducted a similar study using a single per os administration of chlordecone (5 mg/kg) followed 48 hours later by intraperitoneal administration of CCl_4 (0.2 mℓ/kg). Twenty-four hours later the hepatic excretory function of the animals treated with chlordecone and CCl_4 had decreased (20% of controls), while plasma transaminase activities and bilirubin were elevated. Parameters measured or assessed which were not affected by chlordecone pretreatment were: hepatic mixed function oxidase activity, irreversible binding of label from $[^{14}C]CCl_4$ to hepatic protein or lipid, hepatic and renal glutathione concentrations and CCl_4-induced lipid peroxidation of liver tissue measured in vitro and in vivo. The authors conclude that the mechanism for the enhanced toxicity is still unknown; however, the results suggest the interaction between chlordecone and CCl_4 is a subtle one, not causally involving increased covalent binding of the toxin, increased susceptibility of tissue lipids to peroxidative damage or decreased hepatic glutathione.

Curtis et al. (1979b) investigated the effect of pre-exposure to 50 mg/kg photomirex on CCl_4 hepatotoxicity in rats. Photomirex is a photodegradation product of the insecticide mirex, and is also a structural analog of chlordecone. The photomirex/CCl_4 interaction resulted in liver hypertrophy, 7- and 8-fold elevations in SGOT and SGPT over the control rats and rats treated with photomirex alone, and considerable centrilobular necrosis. Liver weight, SGOT and SGPT were unaffected by the CCl_4 challenge alone; therefore, pre-exposure to photomirex does potentiate CCl_4 hepatotoxicity as does chlordecone, a structural analog.

In a review article, Falk (1976) reported on the effects of different chemicals on CCl_4 toxicity. A summary of this review is presented here. Benzo(a)pyrene (BAP) in conjunction with CCl_4 has been shown to enhance tumor production in laboratory animals (Kotin et al., 1962; Proetzel et al., 1964) while CCl_4 alone was found to produce no sarcomas. Weisburger et al. (1965) found that treating rats with acetylaminofluorene (AAF) produced an increase in hepatomas in female rats, from 0% without to 81% with CCl_4. In males, the increase was 73-100%. A change in metabolism of AAF toward greater N-hydroxylation on CCl_4 treatment was found to be the reason for the enhanced tumor incidence (Weisburger and Weisburger, 1963).

Kluwe et al. (1979) found that CCl_4 produced liver damage in male rats fed 100 mg/kg polybrominated biphenyls (PBB) or 200 mg/kg polychlorinated biphenyls (PCB) 28 days before injection of CCl_4, as represented by increased levels of SGOT. The liver damage was greater in the rats fed PBB than in those fed PCB which in turn showed greater damage than the control rats. Functional renal damage was produced by CCl_4 and several other solvents such as TCE and 1,1,2-trichloroethane. Carbon tetrachloride-induced renal dysfunction was found to be potentiated by PBB but not PCB.

Dietz and Traiger (1979) found that pretreatment of rats with either 2-butanone or 2,3-butanediol markedly enhanced the hepatotoxic response to CCl_4 as measured by SGPT and hepatic triglycerides. The administration of CCl_4 and quinalphos (an insecticide) was found to cause morphological changes in the liver, kidney and testes of male rats (Dikshith et al., 1980). The authors suggest that pretreatment of CCl_4 rendered the animals susceptible to the toxic effects of quinalphos.

12.2. ANTAGONISM

As to the antagonistic compounds associated with CCl_4, Hafeman and Hoekstra (1977) report a protective effect of dietary vitamin E, selenium (Se) and methionine against lipid peroxidation induced by CCl_4. In the first of three experiments, 21-day-old male weanling rats were fed a diet deficient in vitamin E and Se and low in methionine. Dietary supplements of these three variables were administered either alone or in combination. To assess the effect of CCl_4, lipid peroxidation was monitored by the evolution of ethane, an auto-oxidation product of ω-3-unsaturated fatty acids. To study the effect of increasing dietary ω-3-unsaturated fat on CCl_4-induced ethane evolution, cod liver oil (CLO), which is rich in ω-3-unsaturated fat, was substituted for lard. A dose of 2 mℓ CCl_4/kg bw was administered via i.p. injection. The investigators found that in all dietary groups, CCl_4 stimulated ethane evolution. However, among mice given dietary supplements of vitamin E, Se and methionine, ethane evolution was reduced 17, 26 and 39%, respectively. The substitution of CLO for lard in the diets resulted in a 6-fold increase in ethane evolution. If only

CCl_4-induced ethane evolution is considered, only dietary supplements of vitamin E and Se resulted in a statistically significant ($p<0.05$) reduction of ethane evolution. It was also noted that among rats given CCl_4 with no supplements there was pronounced mortality which correlated well with ethane evolution. The authors conclude that the toxicity of CCl_4 was decreased in correlation with ethane evolution. Thus, vitamin E, Se and methionine protected against CCl_4-induced lipid peroxidation, probably by maintaining intracellular glutathione and glutathione peroxidase.

In two other experiments, the authors sought to determine the effectiveness of each protective factor alone or in combination and to estimate peroxidation rates in rats in which CCl_4 caused early mortality. In these experiments, a dose of 1 mℓ CCl_4/kg bw was administered via i.p. injection. Results indicated that vitamin E, Se and methionine supplements in all combinations protected against CCl_4 toxicity and that supplements of either vitamin E or Se, or both with methionine, completely prevented CCl_4-induced mortality.

Reserpine, carbon disulfide and diethyldithiocarbamate reportedly diminish the toxic effects of CCl_4 on liver in experimental animals. The mechanisms are unknown, but some of the investigators have speculated that metabolism via the microsomal enzyme system may be involved (Douglas and Clower, 1968; Seawright et al., 1980; Siegers et al., 1978). Chlorpromazine prevented liver necrosis from CCl_4 in short-term rat experiments without affecting lipid peroxidation or binding of CCl_4-reactive metabolites (not identified), but it also lowered body temperatures. The investigators were of the opinion that chlorpromazine only delayed the onset of liver necrosis (Marzi et al., 1980). Cagen and Klaassen (1980) reported that the release of alanine aminotransferase and aspartate aminotransferase into plasma 24 hours following various doses of CCl_4 was markedly lower in rats pretreated for 3 days with zinc chloride (150 μmol/kg/day) than in control rats. Administration of zinc chloride solution was found to increase hepatic concentrations of metallothionein. The authors suggest that metallothionein may protect against CCl_4-induced liver damage by sequestering reactive metabolites of CCl_4.

Seawright and McLean (1967) conducted a study to investigate the decrease in CCl_4 toxicity brought about by a protein-free diet. Male Wistar rats of a Porton strain were orally administered 2.5 mℓ/kg $[^{14}C]CCl_4$ diluted in an equal volume of paraffin oil. Those rats maintained on protein-free diets ≥ 7 days exhibited a decrease in the metabolism of CCl_4 to CO_2 in vivo as well as in microsomal preparations, compared to rats maintained on a stock diet. However, rats injected subcutaneously with 100 mg/kg DDT 1 week before death regained their sensitivity to the toxic effects of CCl_4 as measured by their ability to metabolize CCl_4 and Pyramidon demethylation.

Falk (1976) also reported on the effects of diet on CCl_4 toxicity. A low protein diet fed to rats reduced the hepatic microsomal hydroxylase activity and dramatically reduced the toxicity of CCl_4. The LD_{50} of CCl_4 was 14.7 mℓ/kg on the protein-free diet compared to 6.4 mℓ on a regular diet. When the rats were pretreated with phenobarbital the LD_{50} was 0.5 mℓ/kg.

Mikhail et al. (1978) examined the protective effect of adenosine monophosphate (AMP) on CCl_4 toxicity. Three groups of male and female adult rats were studied: a control group, a group treated with 0.5 mℓ of a 1:1 mixture of CCl_4 in mineral oil/100 g bw via intraperitoneal injection and a group treated with 30 mg AMP via intraperitoneal injection 1/2 hour prior to treatment with CCl_4. The CCl_4 resulted in elevated serum iron, copper, zinc, potassium, sodium and calcium 24 hours after administration. However, pretreatment with AMP led to a normalization of the levels of serum iron, copper and zinc while there were no changes in serum calcium, magnesium, potassium and sodium levels as compared to the CCl_4-treated group. The normalization of zinc may be due to the action of AMP on hormone secretion. Thus, the authors conclude that the normalization of iron and copper may be due to some protective effect of AMP on the liver (Mikhail et al., 1978).

Additional work is reported in the literature dealing with antagonism but will only briefly be mentioned here. Kleczka et al. (1981) determined that an oxidized catechol metabolite rather than the catechol molecule itself may be responsible for the enzymatic inhibition of the activation step of CCl_4 or may interfere with reactions of the $\cdot CCl_3$ radicals formed. The administration of chloramphenicol early in CCl_4 intoxication prevents lipid peroxidation of endoplasmic reticulum membranes in rats (Dolci and Brabec, 1978). Dzhiolev and Balanski (1974) found that CCl_4 injected prior to the administration of urethane reduced the incidence of adenomas by 32 and 47%.

12.3. SUMMARY

Numerous substances have been shown to synergistically affect CCl_4 toxicity. Ethanol has been shown to potentiate CCl_4 toxicity even when the ethanol consumption had taken place prior to exposure. This effect has been documented in case studies and more recently quantified in animal studies. Several other environmental pollutants such as Kepone®, PBB and

PCB have been shown to potentiate CCl_4 toxicity at doses where both substances are not considered toxic. Carbon tetrachloride toxicity has been shown to be inhibited by several compounds, such as chloramphenicol and catechol.

While this information is interesting, it can only be used qualitatively. The probabilities of such synergistic or antagonistic reactions occurring in the natural environment are not available. Once such information is quantified, methods of altering "safe" exposures can be investigated.

13. REGULATIONS AND STANDARDS

13.1. WATER

13.1.1. Ambient Water. In 1980 the U.S. EPA announced availability of 64 ambient water quality criteria documents and associated criteria (Federal Register, 1980b). The criteria associated with the CCl_4 document were 4.0, 0.4 or 0.04 µg/ℓ based on estimated human lifetime cancer risks of 10^{-5}, 10^{-6} or 10^{-7}, respectively. These criteria were derived based on the assumption of a daily contaminated water intake of 2 ℓ and contaminated fish intake of 6.5 g per person. Criteria associated with only the consumption of 6.5 g of contaminated fish were 69, 6.9 or 0.69 µg/ℓ, respectively.

13.1.2. Drinking Water. The NAS recommended "Suggested No-Adverse-Response Levels" (SNARLs) for CCl_4 in drinking water (NAS, 1980). The recommended 24-hour value was 14 mg/ℓ based on data which indicated a toxic effect to the liver of rats 5 hours after one exposure to CCl_4 at 400 mg/kg bw, a 1000-fold safety factor, and an assumed daily water consumption of 2 ℓ for a 70 kg adult. NAS recommended a 7-day SNARL of 2 mg/ℓ based on the assumed cummulative effects of CCl_4 after repeated daily exposure (i.e., 14 mg/ℓ\÷ 7 = 2 mg/ℓ). NAS did not recommend a "chronic exposure" SNARL because CCl_4 is a carcinogen in some animal species.

The Office of Drinking Water (ODW) of the U.S. EPA has recommended draft SNARLs for CCl_4. The 1-day value is 0.2 mg/ℓ based on data which indicate adverse effects in rats after an acute exposure of CCl_4 at 20 mg/kg bw, a 1000-fold safety factor, and an assumed daily water consumption of 1 ℓ for a 10 kg child (Ohanian, 1981). ODW recommended a draft 10-day SNARL at 0.02 mg/ℓ. A longer-term EPA-SNARL was not developed due to the lack of acceptable data on chronic exposure to this compound.

The differences in the NAS and ODW SNARLs are 3-fold: different data bases were used, the NAS-SNARL was calculated for a 70 kg adult rather than a 10 kg child, and the adult was assumed to ingest 2 ℓ of water/day as compared to 1 ℓ/day for a child. Both methods assume 100% absorption of ingested CCl_4.

13.2. AIR

The American Conference of Industrial Governmental Hygienists (ACGIH, 1980) has recommended threshold-limit-values (TLVs) for CCl_4 both as a time-weighted average (TWA) and a short-term-exposure limit (STEL) of 30 and 125 mg/m³, respectively. Previously, ACGIH (1979) recommended values of 65 and 130 mg/m³, respectively. A concise history of other relevant ACGIH recommendations and accompanying logic can be found in the Ambient Water Quality Criteria Document for Carbon Tetrachloride (U.S. EPA, 1980a).

The Occupational Safety and Health Administration (OSHA), U.S. Department of Labor, adopted the American National Standards Institute (ANSI) standard Z37.17-1967 (ANSI, 1967) as the Federal standard for CCl_4 (29 CFR 1910.1000). This standard is 65 mg/m³ for an 8-hour TWA exposure, with an acceptable ceiling exposure concentration of 162.5 mg/m³, and an acceptable maximum peak above the acceptable ceiling concentration for an 8-hour shift of 1300 mg/m³ for 5 minutes in any 4 hours. This adopted ANSI standard was based on human experience and extensive studies on animals. References cited to support it were Adams et al. (1952), Stewart et al. (1961, 1965), Stewart and Dodd (1964), von Oettingen (1964) and Irish (1963).

The National Institute for Occupational Safety and Health (NIOSH, 1975) recommended a CCl_4 TWA value of 12.6 mg/m³ for a 10 hour work day, 40-hour week over a working lifetime. This recommendation was based on liver and eye changes found in workers chronically exposed to CCl_4. In a

1976 revision, NIOSH recommended that the concentration of CCl_4 not be >12.6 mg/m³ of breathing zone air in a 45 ℓ air sample taken over a period not to exceed 1 hour in duration (NIOSH, 1976)

Inhalation standards for various toxic substances in the working environment of several other countries have been published (International Labor Office, 1970). Carbon tetrachloride inhalation standards are shown in Table 13-1. The USSR values are maximum allowable concentrations (MACs) never to be exceeded. Several countries follow USSR standards; others follow the recommendations of ACGIH.

13.3. FOOD

The National Academy of Sciences (NAS, 1978) reported maximum concentrations of CCl_4 permitted in cooked cereal as 50 μg/kg. This value was derived from a FAO/WHO expert committee in 1972. No other information was found concerning guidelines, criteria or standards for CCl_4 in food.

13.4. SUMMARY

Protective levels for CCl_4 in air in the workplace have been suggested by several countries and by several groups within the United States. The number of suggested protective levels demonstrates the wealth of toxicity data in this area. Protective levels for CCl_4 in water (both drinking and ambient) have recently been suggested by the U.S. EPA and NAS. Only one protective level has been suggested for food: that of cooked cereal. Obviously, more work is needed in this latter area.

TABLE 13-1

Carbon Tetrachloride Inhalation Standards of 11 Countries[a]

Country	Standard (mg/m^3)	Qualifications
United States		
ACGIH	30[b]	TWA
	125[b]	STEL
OSHA	65	TWA
	162.5	Ceiling exposure concentration
	1300	Maximum peak of ceiling Concentration not to be exceeded for 5 minutes in any 4 hour period.
NIOSH	12.6[b]	TWA
	12.6[b]	45 ℓ air/60 minutes
Czechoslovakia	50	MAC
	250	Single short exposure
Finland	160	8 hours continuous exposure
Hungary	20	8-hour average
	100	30 minutes
Japan	10	
Poland	20	
Rumania	50	
UAR and SAR	625	
USSR	20	MAC
Yugoslavia	65	

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[a] Source: Adapted from NIOSH, 1975

[b] Recommended standards

14. EFFECTS OF MAJOR CONCERN AND HEALTH HAZARD ASSESSMENT

The assessment of health hazards from CCl_4 requires information which relates specific adverse health effects to dose-exposure conditions. Studies which report the route of exposure, the dose, the duration of exposure, the animal species and the nature of adverse effects are therefore most useful in hazard assessment, involving the prediction of effects from given environmental or occupational exposure situations. Since the route of intake is often important, experimental studies should use the typical routes of human exposure (i.e., ingestion, inhalation or dermal contact). Therefore, studies based on exposure by intraperitoneal or subcutaneous injection are not included.

The discussion of health effects is organized according to the route of exposure. Within each subsection, the discussion first lists the pertinent acute, subchronic and chronic effects, followed by summaries on reproductive, mutagenic and carcinogenic effects. Specific information on dose, exposure, test animal, effects and references are presented in Table 14-1 for acute, subchronic and chronic studies; in Table 14-2 for reproduction studies; in Table 14-3 for mutagenicity studies; and in Table 14-4 for carcinogenicity studies. Note that these tables summarize the toxicity data. Further analysis leading to unit risk estimates for humans is presented in the Appendix.

14.1. PRINCIPAL EFFECTS

Carbon tetrachloride is toxic to humans and animals. Sublethal exposure affects several organs; however, the primary target organs are the liver and the lung. Long-term exposure has resulted in malignant tumors of the liver in three animal species. Several studies have produced satisfactory dose-response information, including estimated "no-effect" levels for humans and

TABLE 14-1

Dose-Related Toxic Effects of Carbon Tetrachloride on Humans and Animals

Route[a]	Animal	Dose[b]	Exposure	Reported Effects[c]	Reference
Ingestion	human	0.06 mg	1 day	Creatinine ROR[d] = 0.17	Sonich et al., 1981
	human	0.11 mg	1 day	Creatinine ROR[d] = 1.25	Sonich et al., 1981
	human	0.18 mg	1 day	Creatinine ROR[d] = 1.74	Sonich et al., 1981
	human	0.21 mg	1 day	Creatinine ROR[d] = 3.39	Sonich et al., 1981
	rat	20 mg/kg	single dose	Liver cell change	Korsrud et al., 1972
	rat	40 mg/kg	single dose	Increased liver fat, increased liver weight, increased liver enzyme levels	Korsrud et al., 1972
	dog	116 mg/kg	single dose	NOEL	Gardner et al., 1924
	rabbit	159 mg/kg	single dose	Liver necrosis	Gardner et al., 1924
	dog	797 mg/kg	single dose	Centrilobular necrosis of liver	Gardner et al., 1924
	mouse	1600 mg/kg	single dose	NOEL	Boyd et al., 1980
	rat	1600 mg/kg	single dose	Increased liver weight and fatty infiltration, some liver necrosis	Murphy and Malley, 1969
	rat	3816 mg/kg	single dose	NOEL	Boyd et al., 1980
	mouse	4000 mg/kg	single dose	Reversible widespread lung cell changes	Boyd et al., 1980
	rat	4000 mg/kg	single dose	Reversible changes in kidney morphology	Striker et al., 1968
	rat	4000 mg/kg	single dose	Behavior changes, diverse lung cell changes, altered liver C-450 content	Gould and Smuckler, 1971
	rat	5088 mg/kg	single dose	Clara cell lesions in lung	Boyd et al., 1980
	rat	22 mg/kg	6 weeks	NOEL	Alumot et al., 1976
	rat	40 mg/kg	6 weeks	Higher levels of total lipids and triglycerides	Alumot et al., 1976
	rat	76 mg/kg	6 weeks	Depressed weight gain, higher levels of total lipids and triglycerides	Alumot et al., 1976

TABLE 14-1 (cont.)

Route[a]	Animal	Dose[b]	Exposure	Reported Effects[c]	Reference
Inhalation	human	63 mg/m^3	3 hours	NOEL	Stewart et al., 1961
	human	309 mg/m^3	1 hour	Decreased serum iron, altered serum transaminase levels	Stewart et al., 1961
	human	290-650 mg/m^3	2 years	Reversible nausea, anorexia, vomiting, discomfort	Kazantis and Bomford, 1960
	rat	300 mg/m^3	24 hours	Reduction in enzyme activity	Merkur'eva et al., 1979
	rat	65 g/m^3	0.3-4.0 hours	Increased liver and kidney weights	Wong and Di Stefano, 1966
	mice	71.8 g/m^3	1 hour	Clara cell lesions in lung	Boyd et al., 1980
	rat	285 g/m^3	0.5 hour	Morphological and cellular changes in lung	Chen et al., 1977
	rat	6.1 mg/m^3	6 weeks[e]	NOEL	Prendergast et al., 1967
	guinea pig	6.1 mg/m^3	90 days[e]	NOEL	Prendergast et al., 1967
	rat	61 mg/m^3	6 weeks[e]	Fatty infiltration and some degeneration of liver	Prendergast et al., 1967
	guinea pig	61 mg/m^3	90 days[e]	3 deaths (of 15 animals), depressed weight gain, liver damage	Prendergast et al., 1967
	rat	515 mg/m^3	6 weeks[e]	Fatty degeneration of liver, morphological changes in liver and lung	Prendergast et al., 1967
	guinea pig	515 mg/m^3	6 weeks[e]	3 deaths (of 15 animals), weight loss, liver damage, morphological changes in liver and lung	Prendergast et al., 1967
	rat	14 mg/m^3	5 hours/day, 5 days/week, 5 months	Changes in hepatic-energy producing processes	Rotenberg, 1978
	rat	315 mg/m^3	10 months[e]	Growth stimulation, NOAEL	Smyth et al., 1936
	guinea pig	315 mg/m^3	10 months[e]	Mortality (9/24) with median 44 exposures, liver cirrhosis	Smyth et al., 1936
	monkey	315 mg/m^3	10 months[e]	Reversible liver degeneration	Smyth et al., 1936
	rat	630 mg/m^3	10 months[e]	Liver cirrhosis after 173 exposures	Smyth et al., 1936

TABLE 14-1 (cont.)

Route[a]	Animal	Dose[b]	Exposure	Reported Effects[c]	Reference
	guinea pig	630 mg/m³	10 months[e]	Mortality (16/24) with median 10 exposures	Smyth et al., 1936
	rat	1260 mg/m³	10 months[e]	Liver cirrhosis after 115 exposures	Smyth et al., 1936
	guinea pig	1260 mg/m³	10 months[e]	Mortality (13/24) with median 3 exposures	Smyth et al., 1936
	monkey	1260 mg/m³	10 months[e]	Reversible liver degeneration, depressed weight gain, sciatic nerve damage	Smyth et al., 1936
	rat	2520 mg/m³	10 months[e]	Growth retardation, liver cirrhosis after 54 exposures	Smyth et al., 1936
	guinea pig	2520 mg/m³	10 months[e]	Mortality (19/24) with median 3 exposures	Smyth et al., 1936
Dermal	guinea pig	1 mℓ/3.1 cm²	0.3 hour	Epidermal spongiosis and karyopyknosis	Kronevi et al., 1979
	guinea pig	1 mℓ/3.1 cm²	16 hours	Pronounced epidermal spongiosis, karyopyknosis and junctional separation, marked hydropic changes in liver, liver necrosis	Kronevi et al., 1979

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[a] Oral exposure includes gavage, drinking water and diet.

[b] Oral exposure is in mg CCl_4 or mg of CCl_4/kg body weight. Inhalation exposure is in weight of CCl_4/cubic meter of air. When ppm is supplied by the referenced article, conversion is 6.5 mg/m³ = 1 ppm. Dermal exposure is volume of CCl_4 per area of skin. These are the reported animal exposure levels and are not human equivalent exposure levels. Therefore, doses are not comparable and may not be directly applicable to the human exposure situation.

[c] The type of effect level (NOEL, etc.) was determined for this document and was not necessarily reported by the original authors.

[d] ROR is the ratio of odds ratios (see text, Section 8.2.2.). Corresponding dose is estimated for drinking water by assuming that the Cincinnati ratio of drinking water concentration to river water concentration (1:2) is constant for all cities in the study. A test for linear trend of ROR on dose is significant ($p<0.05$).

[e] Exposure: 8 hours/day, 5 days/week

TABLE 14-2

Reproductive Effects of Carbon Tetrachloride from Subchronic Exposure

Route	Dose Level and Species	Exposure Duration	Biological Endpoint	Reference
Inhalation	622 mg/kg rat	Daily; days 6-15 of gestation	Retarded fetal development	Schwetz et al., 1974
Inhalation	475 mg/kg rat	Daily; for three generations	Reduced fertility	Smyth et al., 1936
Oral	2370 mg/kg rat	2 or 3 days; during gestation	Reduced fertility	Wilson, 1954

TABLE 14-3

Summary Table for Mutagenicity Studies

Assay	Response	Reference
Ames test	negative	McCann et al., 1975
E. coli reversion test	negative	Uehleke et al., 1976
In vitro chromosome assay	negative	Dean and Hudson-Walker, 1979
Yeast cells	positive	Callen et al., 1980

TABLE 14-4

Carcinogenicity Studies Useful for Risk Assessment of Carbon Tetrachloride[a]

Species/Strain	Number[b]	Dose Regimen[c]	Dose	Duration		Animals with Tumors		Tumor Type	Reference
				Exposure	Observation	Control	Treated		
Mice/inbred L	73 treated 152 control	2-3 times/week in olive oil	0.04 mℓ (~2100 mg/kg)	4 months	7.0-7.5 months	1%	47%	Liver tumor	Edwards et al., 1942
Mice/B6C3F_1	200 treated 40 control	5 times/week in corn oil	1250 mg/kg 2500 mg/kg	78 weeks	92 weeks	8%	100% 97%	Hepatocellular carcinoma	NCI, 1976a,b
Rats/Osborne-Mendel	168 treated 37 control	5 times/week in corn oil	47 mg/kg (males) 94 mg/kg (males) 80 mg/kg (females) 159 mg/kg (females)	78 weeks	110 weeks	0%	4% 4% 9% 3%	Hepatocellular carcinoma	NCI, 1976a,b
Hamsters/Syrian Golden	19 treated 80 control	1 time/week in corn oil	7.7 μℓ (12.42 mg)	30 weeks	55 weeks	0%	53%	Liver-cell carcinoma	Della Porta et al., 1961

[a] Studies with sufficient dose/response information and adequate study design

[b] Includes males and females

[c] By gavage or gastric intubation

four animal species. These studies are classified as satisfactory in that they not only provide all of the necessary quantitative information (e.g., number of animals in control and treated groups), but also include sufficient detail to demonstrate the high quality and defensibility of the research.

14.1.1. **Ingestion.** The only dose-related human data with complete quantitative information for oral ingestion (see Section 8.8.2.) are from an epidemiological study which found elevated serum creatinine levels following an estimated 1-day exposure to CCl_4 in drinking water. Several animal studies showed dose-related effects from CCl_4 ingested via water, gavage or diet. Most of these studies involved a single dose (e.g., Korsrud et al., 1972). Acute effects (with increasing dose) included greater liver weight than normal, greater amount of liver fat, changes in enzyme levels or activity, some liver necrosis, reversible lung and kidney structural changes, lung lesions and behavioral abnormalities. One short-term (6-week) study in rats showed dose-related increases in total serum lipids and triglycerides and depressed weight gain (Alumot et al., 1976).

Several authors reported that CCl_4 does not appear to be a teratogen but that it can affect reproduction following subchronic exposure. Oral administration of CCl_4 to rats for either 2 or 3 days during days 7-14 of gestation was reported to cause a reduction in litter sizes and an increase in the number of fetal resorptions (Wilson, 1954).

Carbon tetrachloride has produced liver tumors in hamsters, mice and rats. A number of experiments have been conducted using mice of various strains ($B6C3F_1$, C3H, A and L) and different dosage regimens of CCl_4 (Edwards et al., 1942; NCI, 1976). The types of tumors observed have included hepatomas, hepatocellular carcinomas and hyperplastic hepatic nodules. Hyperplastic hepatic nodules were also induced in Syrian golden hamsters by oral doses of CCl_4 (Della Porta et al., 1961). Effects induced by CCl_4 in several strains of rats also include cholangiofibrosis, hepatic hyperplasia, hyperplastic hepatic nodules and hepatic carcinomas (Reuber and Glover, 1967, 1970).

14.1.2. **Inhalation.** The majority of the toxicity studies encountered in the available literature involved inhalation of CCl_4 and included a wide range of exposure levels. One 70-minute exposure study found decreased serum iron and altered serum transaminase levels, implicating liver damage (Stewart et al., 1961). A 2-year exposure in humans showed reversible nausea, anorexia, vomiting and epigastric discomfort (Kazantis and Bomford, 1960).

Effects from short-term exposure on animals appear to be dose-dependent and include: increased kidney and liver weights, reduction in activities of various enzymes, morphological and cellular changes in the liver and the lung, and Clara cell lesions in the lung. Dose-related effects from subchronic exposures are highly species-specific. For example, reversible liver damage was seen in the monkey at the same dose and duration which caused liver cirrhosis and increased mortality in the guinea pig. Other effects due to subchronic exposures include minor liver damage, weight loss and damage to the sciatic nerve.

Only one chronic inhalation animal study (of 10 months duration) was found in the literature. Liver damage was reported. The authors did not report liver tumors (Smyth et al., 1936).

When pregnant rats were exposed to CCl_4 by inhalation on days 6-15 of gestation, the exposure resulted in decreased fetal body weights and lengths (Schwetz et al., 1974). In a three-generation inhalation study involving rats, a dose of 238 mg/kg/day did not affect reproductive functions, while a dose of 475 mg/kg/day caused reduced litter sizes (Smyth et al., 1936). Single subcutaneous injection of pregnant animals with CCl_4 has also been reported to produce histological and biochemical changes in the livers of the offspring; thus, Bhattacharyya (1965) concludes that CCl_4 may be fetotoxic and can cross the placental barrier.

14.1.3. Dermal Exposure. Guinea pigs exposed to pure CCl_4 in a skin painting experiment showed epidermal spongiosis and karyopyknosis, altered liver morphology and some liver necrosis (Kronevi et al., 1979).

14.1.4. Mutagenicity. Carbon tetrachloride has produced negative results in the Ames Salmonella test, in both the presence and the absence of microsomal activation (McCann et al., 1975) and in the Escherichia coli K12 test (Uehleke et al., 1976). Carbon tetrachloride produced negative results and was nonclastogenic in a chromosome assay using an epithelial-type cell line derived from rat liver which possessed intrinsic metabolizing activity (Dean and Hudson-Walker, 1979).

However, Callen et al. (1980) found CCl_4 to be genetically active and cytotoxic in strain D yeast cells of Saccharomyces cerevisiae. In these cells, which contain a cytochrome P-450-dependent mono-oxygenase system, CCl_4 caused increased frequencies of "gene conversion and mitotic recombination" and decreased cell survival.

14.2. SENSITIVE POPULATIONS

Studies on human sensitivity are limited. There is clinical evidence, discussed in Chapter 12, that isopropanol and ethanol may potentiate CCl_4 toxicity in humans. As described by Moon (1950), a repeated history of alcoholism in cases of fatal CCl_4 poisoning may indicate a synergistic effect between alcohol and CCl_4. In other case reports, very obese and undernourished persons suffering from pulmonary diseases, gastric ulcers, liver or kidney diseases, diabetes or glandular disturbances seem especially sensitive to the toxic effects of CCl_4.

Supportive studies on rats suggest that older animals are more susceptible to the toxic effects of CCl_4 than are younger animals, and that males are more susceptible than females (Rueber and Glover, 1967). Chaturvedi (1969) examined age and sex as factors in CCl_4 toxicity. The findings revealed that female rats are less susceptible to the adverse effects of different hepatotoxic agents. Chaturvedi (1969) postulated that this was due to different hormonal and enzyme patterns and the lack of certain proteins in the female liver. The sex difference noticed in adult rats was not as apparent in young rats.

Nutritional status may also affect the degree of toxicity following exposure to CCl_4 in rats. Gyorgy et al. (1946) exposed young rats on various diets to ~300 mg/kg CCl_4 in a gas chamber 7 hours/day, 5 days/week for 45 months. Animals were then sacrificed, and histopathological effects in the liver and kidneys were determined. When animals fed standard chow were compared to other groups, these effects were more severe in animals fed a diet high in lipid and low in carbohydrate, or a diet low in protein. Methionine appeared to protect against increased toxicity, particularly kidney damage, caused by low-protein diets (Gyorgy et al., 1946).

The interaction of CCl_4 with other chemicals has resulted in an enhancement of the toxic effects produced in animals by either chemical alone. Exposure of animals to selected environmental carcinogens in combination with CCl_4 has resulted in an increase in carcinogenicity. In addition, certain chemicals appear to increase the toxic effects of CCl_4 on the liver and other organs of experimental animals.

14.3. QUALITATIVE HEALTH HAZARD ASSESSMENT

The assessment of human health risks, that is, the likelihood of certain adverse effects from given exposure scenarios, is hampered by the paucity of good dose-response data in humans. Of the three human studies discussed above, the acute epidemiological study involving oral ingestion (Sonich et al., 1981) and the acute inhalation study (Stewart et al., 1961) show serum alterations. The short-term inhalation study shows reversible, minor CNS and gastrointestinal effects (Kazantis and Bomford, 1960). In other instances, although several case reports document human effects, concentrations and exposures are not reported. Therefore, the prediction of toxic effects in humans and the determination of no-effect levels for subsequent use in a quantitative hazard or risk assessment, are primarily estimated from the many animal studies showing dose-related effects.

The major reported sublethal health hazards to humans from exposure to CCl_4 are damage to the liver, lungs, kidneys and central nervous system. Less severe adverse effects include altered enzyme activities following ingestion and inhalation, gastrointestinal disturbances following inhalation, and epidermal damage following dermal contact. In animal studies, some effects are seen in areas distant from the contact interface. These include lung damage from oral ingestion, liver damage from inhalation and from dermal contact and, to a lesser degree, enzyme disturbances from inhalation and ingestion.

Teratogenic, mutagenic and carcinogenic effects have not been demonstrated in humans, and only carcinogenicity has been shown in experimental animals. In addition, fetotoxicity and neonatal toxicity have been shown in rats (Schwetz et al., 1974; Bhattacharyya, 1965). Reproductive efficiency has also been affected in rats evidenced by reduced litter sizes (Smyth et al., 1936). Negative mutagenicity has been demonstrated in four studies including Salmonella typhimurium (McCann et al., 1975; Simmon and Tardiff, 1977), E. coli (Uehleke et al., 1976), and in a recently developed epithelial-type cell chromosome assay (Dean and Hudson-Walker, 1979). The single positive mutagenicity test in yeast cells, Saccharomyces cerevisiae, therefore requires further confirmation (Callen et al., 1980).

Carcinogenicity has been demonstrated in three experimental animal species, but predominantly several strains of rats and mice (NCI, 1976a,b; Edwards et al., 1942; Reuber and Glover, 1970). Thus, CCl_4 should be considered a potential human carcinogen, even though no definitive cause-and-effect human data exist.

14.3.1. Animal Toxicity Studies Useful for Hazard Assessment. The preferred studies for hazard assessment are those which provide definite effect levels. Adverse effects are defined here as functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge. Adverse effects which are not carcinogenic are assumed to be threshold phenomena. The threshold region of toxicity is estimated by evaluating four types of effect levels:

NOEL	No-Observed-Effect Level: That exposure level at which there are no statistically significant increases in frequency or severity of effects between the exposed population and its appropriate control.
NOAEL	No-Observed-Adverse-Effect Level: That exposure level at which there are no statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects are produced at this level, but they are not considered to be adverse.
LOAEL	Lowest-Observed-Adverse-Effect Level: The lowest exposure level in a study or group of studies which produces statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

FEL Frank-Effect Level: That exposure level which produces unmistakable adverse effects, ranging from reversible histopathological damage to irreversible functional impairment or mortality, at a statistically significant increase in frequency or severity between an exposed population and its appropriate control.

Studies providing both a NOAEL and a LOAEL, therefore, are the most useful studies for hazard assessment.

The rat was the most commonly used animal in dose-response studies on CCl_4, with 12 satisfactory studies (as defined earlier) representing 22 dose-exposure groups (see Table 14-1). Korsrud et al. (1972) reported a single-dose LOAEL for rats of 20 mg/kg, virtually identical to the 6-week rat NOEL of 22 mg/kg given by Alumot et al. (1976). These results may be consistent. The infrequent, minor liver cell changes reported by Korsrud et al. may have been present in the Alumot et al. animals without affecting the serum levels monitored in the latter study. This illustrates the dependence of effect-level category on the endpoint investigated. Extensive single-dose information on lung effects from oral ingestion was reported by Boyd et al. (1980), who studied two dose levels in both rats and mice, the lower in each case resulting in a "lung NOEL." Other studies which provide dose-related effect data for single oral exposures are listed in Table 14-1. The only subchronic oral study (6 weeks) provided a NOEL and two higher adverse effect levels for rats (Alumot et al., 1976).

Acute inhalation studies did not provide dose-related effect data. Intercomparison of four studies in rats showed a dose-dependent progression in severity of effects, with doses ranging from 300-285,000 mg/m³ (Merkur'eva et al., 1979; Wong and DiStefano, 1966; Boyd et al., 1980; Chen et al., 1977). Useful subchronic inhalation data were provided by Prendergast et al. (1967), who used three dose levels (6.1-515 mg/m³) including a

NOEL, resulting in progressive effects on the liver of rats. The authors also applied identical dose levels to guinea pigs, the lowest also being a NOEL, with more severe effects resulting from the two higher levels. The only chronic inhalation study involved rats and guinea pigs and demonstrated effects from four dose levels administered for 10 months (Smyth et al., 1936). The effects in rats were predominantly on the liver, whereas in the guinea pigs the endpoint was mortality, dependent on dose and length of exposure.

The only dermal study providing dose-response information involved a single dose for short durations (0.3 and 16 hours) using guinea pigs (Kronevi et al., 1979). This study is most important for the duration-related effects on the liver, demonstrating definite effects of dermal exposures at a distant site.

14.3.2. Animal Carcinogenicity Studies. Liver tumors have been demonstrated in several species following oral exposure to CCl_4. Only a few studies, however, were designed to demonstrate the relation between tumor incidence and dose or exposure duration. No suitable inhalation studies with dose-response cancer data were located in the available literature. Three oral studies provided adequate information on dose and incidence in controls and treated animals (see Table 14-4). Edwards et al. (1942) reported dose-related incidence of tumors for inbred strain L mice of two age groups, NCI (1976a,b, 1977) demonstrated significant tumor incidence for Osborne-Mendel rats and treated $B6C3F_1$ mice, and Della Porta et al. (1961) showed significant tumor incidence in Syrian golden hamsters. It should be noted that in most of the animal studies showing carcinogenicity either the length of the study was too short, the incidence rate was too high, or survival was too poor for a dose-response estimation for lifetime exposure

(NAS, 1978). The National Research Council recognizes this problem and uses the rat NCI (1976a,b) bioassay for trichloroethylene (with CCl_4 as a positive control) for determining a carcinogenic risk estimate for CCl_4.

14.4. FACTORS INFLUENCING HEALTH HAZARD ASSESSMENT

14.4.1. Exposure. Carbon tetrachloride is persistent in air and ground water. Contamination of surface water and soil by CCl_4 is not likely to present long-term hazards due to its rapid volatilization. However, large quantities of CCl_4 in bodies of cold water, such as lakes, are likely to remain submerged and be relatively stable, contaminating the body of water for several years. Carbon tetrachloride is readily absorbed through the lungs, but it is also rapidly exhaled; it is excreted through all routes, predominantly as the parent compound. The relative contributions of these factors to the bioavailability and body burden of CCl_4 in humans are not well defined. Also, experimental animals may differ substantially from humans in terms of oral and dermal efficiencies of absorption. Thus, application of human or animal pharmacokinetic information to quantitative human health hazard assessment does not seem feasible at this time. Consequently, the usable exposure information is from monitoring data of ambient CCl_4 levels in air, water and other liquids, and food.

14.4.2. Estimated Threshold No-effect Levels.* The dose-response data for CCl_4 are quite limited, especially regarding effects on humans (Table 14-5). A human NOAEL for oral ingestion was reported as 0.2 mg/day (~0.1 ppm) for 1-day exposure. The observed effect was a dose-related increase in the frequency of elevated creatinine levels in the study population.

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*Animal studies discussed herein indicate that CCl_4 is a potential human carcinogen, and hence a no-effect level may not exist. These no-effect levels are supplied for comparison purposes.

A human NOEL for inhalation was reported as 63 mg/m³ (~10 ppm) for 3-hour exposure. The monitored effects were serum enzyme and iron levels. Animal data are slightly more complete, with rat NOELs (not human equiva- lent) for inhalation ranging from 6.1-315 mg/m³, depending on length of exposure. Insufficient information exists to allow estimation of NOAELs based upon long-term exposures.

In light of the uncertainties and inadequacies associated with the data base for CCl_4, particularly with the human NOEL and NOAEL given above, calculations of acceptable chronic exposure levels must be approached with caution. The lack of good absorption data is the main obstacle to accurate conversion of the animal exposure data to human equivalent exposures.

14.4.3. Carcinogenicity. Liver tumors have been shown to result from oral exposure to CCl_4 by three species of animals. Although several authors note that toxic effects are concurrent with liver tumors, it has not been established that tissue damage is a necessary precursor to CCl_4 carcinogenesis. The estimation of carcinogenic risk (Appendix) is based on the assumption of low-dose linearity, which is supported by studies on mutagenesis. The inconclusive nature of the presently available mutagenicity evidence for CCl_4 then adds considerable uncertainty to any risk estimation based on low-dose extrapolation.

TABLE 14-5

Reported No-Effect Levels for Toxicity of Carbon Tetrachloride[a]

Route	Exposure	Animal	No-Effect Level[b]	Lowest-Observable-Adverse-Effect Level[c]	Observed Effects	Reference
Oral	single dose	rat	d/	20 mg/kg	Liver cell changes	Korsrud et al., 1972
		mouse	1600 mg/kg	4000 mg/kg	Widespread lung cell changes	Boyd et al., 1980
		dog	116 mg/kg	797 mg/kg	Liver necrosis	Gardner et al., 1924
	acute	human[e]	0.2 mg/day	--	Elevated serum creatinine	Sonich et al., 1981
	subchronic	rat	22 mg/kg	40 mg/kg	Elevated serum lipids and triglycerides	Alumot et al., 1976
	chronic	rat	10 mg/kg	--	Reproductive performance and body weight	Alumot et al., 1976[f]
Inhalation	acute	human	63 mg/m^3	309 mg/m^3	Decreased serum iron and altered transaminase levels	Stewart et al., 1961
	subchronic	rat (6 weeks)	6.1 mg/m^3	61 mg/m^3	Liver degeneration and fatty infiltration	Prendergast et al., 1967
		rat (5 months)	14 mg/m^3	--	Altered respiratory rates and sensitivity of respiratory enzymes to inhibitors	Rotenberg, 1978
		guinea pig	6.1 mg/m^3	61 mg/m^3[e]	Increased mortality, liver damage	Prendergast et al., 1967
	chronic	rat	315 mg/m^3	630 mg/m^3	Liver cirrhosis	Smyth et al., 1936

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[a] Extracted from Table 14-1

[b] Includes no-observable-effect levels and no-observable-adverse-effect levels (see Table 14-1). These are the reported animal exposure levels and are not human equivalent exposure levels. Therefore, doses are not comparable and may not be directly applicable to the human exposure situation.

[c] Lowest or only adverse-effect level reported

[d] No NOEL was reported for the rat in this study. Boyd et al. (1980) gives a rat NOEL of 3816 mg/kg; however, this is for lung effects only.

[e] The elevated serum creatinine levels are not considered to be an adverse effect. No human data exist showing dose-related, adverse effects from oral ingestion of CCl_4.

[f] One-year results inferred from a 2-year study; see discussion in Section 8.1.3.

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APPENDIX

Unit Risk Estimates for Cancer

DEFINITION

Unit risk is one index of the relative carcinogenic potential of a chemical. Unit risk is defined here as the lifetime risk to humans of contracting cancer from a daily exposure to a concentration of 1 μg/ℓ in water via ingestion or a daily exposure to 1 μg/m³ in air via inhalation. The main assumptions for such risk estimates are 70 kg bw, 2 ℓ/day consumption of water and 20 m³/day inhalation rate (ICRP, 1975). The unit risk represents only the estimated risk at the stated exposure concentrations. It should not be interpreted as the slope at any exposure level since the shape of the curve in the low-dose region is not known.

The unit risk estimate for CCl_4 represents an extrapolation below the dose-risk range of experimental data. There is currently no solid scientific basis for any mathematical extrapolation model that relates exposure to cancer risk at the extremely low concentrations, including the unit concentration given above, that must be dealt with in evaluating environmental hazards. For practical reasons the correspondingly low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. Low-dose extrapolation must, therefore, be based on current understanding of the mechanisms of carcinogenesis. At the present time the dominant view of the carcinogenic process involves the concept that most agents that cause cancer also cause irreversible damage to DNA. This position is based in part on the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal response that is characteristic of mutagenesis is associated with a linear non-threshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at higher doses, there can be an upward curvature probably reflecting the effects of multistage processes on the mutagenic response. The linear non-threshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxin in the diet). Some supporting evidence also exists from animal experiments (e.g., liver tumors induced in mice by 2-acetylaminofluorene in the large scale ED_{01} study at the National Center for Toxicological Research and the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because it has the best, albeit limited, scientific basis of any of the current mathematical extrapolation models, the non-threshold model which is linear at low doses has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with such a model should be regarded as conservative, representing the most plausible upper-limit for the risk, i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation chosen to describe the dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data. It is constrained to ensure linearity in the low dose region at least for the upper confidence limit by requiring non-negative values for the fitted coefficients. Furthermore, there exists a procedure for estimating an upper confidence limit on the slope at low extrapolated doses that is based on fitting the data at all experimental dose levels. Other dose-response models have been proposed which are also linear in the low dose region. The procedure recommended by the Carcinogen Assessment Group of EPA, however, involves estimating a most plausible upper limit of the slope at low doses. The other models (discussed later) can be shown to give lower slopes for the same data set than does the linearized multistage model, when extrapolated to the low dose region. Thus, the extrapolation model preferred by the Carcinogen Assessment Group is the multistage model.

EXPERIMENTAL STUDIES USED IN UNIT RISK ESTIMATES

Three oral studies on animals have sufficient information to allow estimation of unit risk. The oral studies are the positive control data for mice and rats used in three of its bioassays (NCI, 1976a,b, 1977), the Edwards et al. (1942) mice data and the Della Porta et al. (1961) hamster data. The incidence data and other pertinent quantitative information on these studies are presented in Table 1. For all studies, male and female data were combined. This was done because of the small sample sizes in the groups segregated by sex. No appropriate inhalation studies or human oral studies were found in the available literature.

Each of these oral studies has one or several characteristics which make it less than ideal for risk estimation for continuous daily exposure over a lifetime. Della Porta et al. (1961) did not report results for a control group, although they did report the incidence rate for vehicle controls in a different study. Moreover, the dose was administered only once per week and was reduced by half after 7 weeks, forcing the use of a time-weighted average approximation to a daily dose. The sample size (19) was also small. Edwards et al. (1942) exposed the mice for only 4 months and observed them

TABLE 1

Data for Cancer Studies Used in Unit Risk Calculations

Animal	Average Daily Dose	Responders/ Tested	Other Data	Reference
Hamster	0	0/80	Average bw: 0.108 kg Exposure: 30 weeks Experiment: 55 weeks Assumed lifespan: 55 weeks	Della Porta et al., 1961
	0.95 mg	10/19		
Mouse	0	2/52	Average bw: 0.03 kg (assumed) Exposure: 17 weeks Experiment: 31 weeks Assumed lifespan: 78 weeks	Edwards et al., 1942
	15 mg	34/73		
Mouse	0	6/157	Average bw: 0.028 kg Exposure: 78 weeks Experiment: 92 weeks Assumed lifespan: 92 weeks	NCI, 1976a,b, 1977
	21 mg	89/89		
	42 mg	90/93		
Rat (M,F)	0	0/37	Average bw: 0.45 kg Exposure: 78 weeks Experiment: 110 weeks Assumed lifespan: 110 weeks	NCI, 1976a,b, 1977
(M)	11 mg	2/45		
(F)	18 mg	4/46		
(M)	21 mg	2/47		
(F)	36 mg	1/30		

M = male, F = female

for ~8 months, much less than the desired lifetime experiment (1.5-2 yrs). The ages varied; the animals initially ranged from 2.5-7.5 months of age. In the NCI (1976a,b, 1977) study on mice, both low and high dose groups showed virtually 100% response (89/89 and 90/93, respectively), so that no information was available on the slope of the dose-response curve. As a consequence, risk projections for doses lower than those used in the study will be underestimated by an unknown amount. In the NCI (1976a,b, 1977) study on rats, survival to the end of exposure was poor, the dose was changed forcing use of a TWA dose estimate, and when segregated by sex, the sample sizes were small so that only the low-dose females were statistically significantly different from controls. The combining of the male and female rats when different, though similar, doses were used (see Table 1) may add further uncertainty.

Insufficient metabolic and pharmacokinetic data precluded the selection of the most appropriate species for use in estimating human risk. Because no study could be selected as "best" and "most appropriate", all four data sets are used in separate estimates of unit risk. In addition, an average unit risk estimate is also calculated which is the geometric mean of the four separate estimates.

DESCRIPTION OF THE PREFERRED LOW-DOSE ANIMAL EXTRAPOLATION MODEL

Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where all coefficients (q_0, q_1 ...q_k) are non-negative. The unit risk estimates are based on excess or extra risk over the background rate at dose d, i.e. the effect of treatment:

$$P_t(d) = \frac{P(d) - P(o)}{1 - P(o)}$$

It follows that

$$P_t(d) = 1 - \exp\left[-(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right].$$

The point estimate of the coefficients q_i, $i = 0, 1, 2, \ldots, k$, and consequently the extra risk function $P_t(d)$ at any given dose d, is calculated by maximzing the likelihood function of the data. The point estimate and the 95% upper confidence limit of the extra risk $P_t(d)$ are calculated by using the computer program GLOBAL 79 developed by Crump and Watson (1979). The upper confidence limit for the extra risk calculated at low doses is always linear with dose. This is conceptually consistent with the linear non-threshold concept discussed earlier. The slope parameter q_1^* is taken as an upper bound (at low doses) of the potency of the chemical in inducing cancer.

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment including the control group.

Whenever the multistage model does not fit the data sufficiently well, the data point at the highest dose is deleted and the model is refitted to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i\,(1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of dose groups. The fit is determined to be unacceptable whenever X^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

CONSIDERATIONS IN SELECTING INCIDENCE DATA

The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is statistically significantly higher than the control for at least one test dose level and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. Usually, the conservative approach adopted by the Carcinogen Assessment Group selects the data set which gives the highest estimate of the unit risk for humans. Because of the difficulties with each CCl_4 study described earlier, no selection was deemed appropriate. Instead, the separate estimates and their geometric mean are presented.

If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

DESCRIPTION OF THE PREFERRED METHOD FOR CALCULATING HUMAN EQUIVALENT UNIT RISK

The method adopted by the Carcinogen Assessment Group for calculating a human equivalent estimate of unit risk from animal data employs two adjustments (Federal Register, 1980b) reflecting species differences and the influence of exposure duration on lifetime cancer risk. First, the animal doses are expressed as the time-weighted-average (TWA) daily dose over the duration of the experiment, and the low-dose extrapolation model is fitted to the resulting dose-incidence data. The risk is then estimated for a daily dose of 2 μg/day, which is the oral daily exposure corresponding to a water concentration of 1 μg/ℓ and human intake of 2 ℓ/day. This risk is then multiplied by each of the above adjustment factors to obtain the human equivalent oral unit risk.

The models used to develop the species and duration adjustment factors represent the best scientific judgment based on available data. Other approaches have been suggested and are discussed in a later section.

The preferred model for equitoxic dose across species, or equivalently for risk at a constant dose across species, is based on an adjustment for metabolic diffferences. Metabolic rate has been suggested to be roughly proportional to body surface area (Mantel and Schneiderman, 1975; Calabrese, 1982), thus the equitoxic model is dose/surface area = constant. Equating the animal and human ratios and solving for the human dose gives:

$$d_h = d_a\ (S_h/S_a)$$

where d is daily dose (mg/day), S is surface area, and a and h refer to animal and human, respectively. The surface area is roughly proportional to the 2/3 power of body weight, and the proportionality constant is approximately the same (~10) for a variety of species. The human dose is then approximated by

$$d_h = d_a\ (W_h/W_a)^{2/3}$$

The unit risk estimate represents the lifetime risk for lifetime exposure to the carcinogen. When the animal experiment is partial lifetime, an adjustment is necessary to allow for positive responses that would have occurred had sufficient time been allowed for the tumors to develop. The risk is then adjusted upward (or equivalently the dose downward) to reflect the missing responders in the short experiment. The adjustment coefficient is $(L/L_e)^3$, where L is the animal lifespan and L_e is the duration of the experiment. The exponent 3 is supported in part by Doll (1971), who showed that age-specific rates for humans increase by at least the second power of age, thus the cumulative tumor rate should increase by at least the third power of age. The choice of 3 for the exponent is also supported by Druckrey (1967) who showed that for a constant incidence rate, the dose-duration relationship was represented by dt^n = constant with n ranging from 2-4 in his experiments. With n=3, Druckrey's results suggest that the dose used for half-lifetime exposure (and observation) can be reduced to one-eighth its value for lifetime exposure and the incidence rate will be the same. Druckrey's results then reflect the influence of both exposure duration and observation period on the resulting incidence rate.

This adjustment factor is consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Crump (1979, 1982) where the probability of cancer by age t at dose d is

$$P(d,t) = 1 - \exp[-f(t)*g(d)].$$

For comparison, Crump's (1982) time-to-tumor model is also used to estimate unit risk for the NCI rat and mice studies (NCI, 1976a,b, 1977) which had time-to-tumor data. In the above model, g(d) is the multistage polynomial, and f(t) is $(t-t_o)^k$, where t_o may be interpreted as minimum induction time.

Interpretation of Quantitative Estimates

For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification regarding carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors. The exposure levels used in the unit risk calculations (1 μg/ℓ, 1 μg/m³) correspond to estimated intake of 2 μg/day via ingestion and 20 μg/day via inhalation. The expected human intake rates for CCl_4 are in the same range: 4 μg/day from food, 9 μg/day from fluids and 13 μg/day from air (see Chapter 4). The assumed dose-response curve is quite linear in this dose range so that the risk is proportional to exposure level, i.e., the upper limit estimate of risk is the unit risk multiplied by the exposure concentration.

The unit risk estimate can give a rough indication of the relative potency of a given agent compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain and sex, and by the same route of exposure. For unit risk estimates for air, the preferable studies would use exposure by inhalation.

The quantitative aspect of the carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process (e.g., setting regulatory priorities, evaluating the adequacy of technology-based controls, etc.). However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. At best, the low-dose linear extrapolation model used here provides a rough, but plausible estimate of the upper-limit of risk, i.e., it is not likely that the true risk would be much more than the estimated risk, but it could very well be considerably lower. The risk estimates presented in subsequent sections should not be regarded as accurate representations of the expected cancer risks even when the exposures are accurately defined. The estimates presented may be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

Unit Risk Estimates for Ingestion Exposure

The unit risk estimates based on human equivalent doses as discussed above are given in Table 2. Since the NCI studies (1976a,b, 1977) on rats and mice were the only ones to present time-to-tumor data, they are the only data sets evaluated using the time-to-tumor model. Both the maximum likelihood estimates (MLE) and upper 95% confidence limits are presented, as well as their geometric means. For the NCI (1976a,b) mouse data, the goodness-of-fit criterion was not satisfied ($x^2 = 14.4$) for the multistage (and one-hit) model. The risk estimates are presented anyway since the model cannot be fitted to the data if the high dose group is deleted, due to the 100% response at the low dose. Because of the protective approach discussed earlier which led (in part) to the adoption of the multistage model, and because the MLE does not account for estimation errors due to small sample sizes, the 95% upper limit on risk is preferred. Furthermore, since no study was entirely adequate for risk assessment purposes, the geometric mean of the upper confidence limits is preferred as the most plausible upper limit estimate of unit risk. For lifetime ingestion of 2 ℓ/day of water, the recommended (based upon present information and current understanding of carcinogenesis) estimate of unit risk (concentration of 1 µg/ℓ) is 3.7×10^{-6}.

ALTERNATIVE METHODOLOGICAL APPROACHES

The methods described above, which have been adopted by the Carcinogen Assessment Group, are consistently conservative, i.e., tending toward high estimates of cancer risk. The aspect which contributes most to this conservatism is the choice of the linearized multistage model for low-dose

TABLE 2

Human Equivalent Unit Risk Estimates for Ingestion Exposure with Specific Adjustments[a]

Data Set	Extrapolation Model			
	Multistage[b]		Time-to-Tumor[b]	
	MLE	UL	MLE	UL
Della Porta et al. (1961)	2.1E-5	3.4E-5	c/	
Edwards et al. (1942)	7.1E-6	9.4E-6	c/	
NCI (1976) mouse	1.4E-6	1.8E-6	1.8E-6	2.2E-6
NCI (1976) rat	1.9E-7	3.1E-7	3.1E-7	5.3E-7
All (geometric mean)	2.5E-6	3.7E-6	7.5E-7	1.1E-6

[a] For ingestion of 1 μg CCl_4 per ℓ water daily for life. Species conversion uses dose/body surface area. Duration adjustment for partial lifetime experiment used for Edwards et al. (1942) study is (fraction lifespan)$^{-3}$.

[b] MLE = Maximum likelihood estimate; UL = upper 95% confidence limit.

[c] No time-to-tumor data were available for these studies.

extrapolation. Other extrapolation models have been suggested, and are included below for comparison. These other models generally give lower estimates of risk than does the multistage model.

The various adjustment factors can also be calculated in different ways. The uncertainties related to the several models and adjustment factors and their influences on the risk estimates are discussed below. Generally, most of the uncertainty in estimating cancer risk from animal data is due to the limited data available in the bioassays, especially due to the high dose levels used, so that almost nothing is known about the shape of the dose-response curve at low doses or about the differences in low-dose incidence rates across species.

Low-Dose Extrapolation Models

Four models are used to extrapolate from the region of the experimental ingestion data to the levels corresponding to 1 μg/ℓ (Albert, 1983). All of these models relate exposure level to the incidence of tumor-bearing animals (Table 3). The "linearized" multistage model is constrained to have non-negative parameter values, and has the same number of parameters as the number of dose groups (including the control group). The one-hit model has two parameters. These two models are linear at sufficiently low doses. The Weibull model has three parameters. When only two dose groups exist, the Weibull exponent is set at 1, and then the Weibull model is also linear at low doses. The log probit model is used to represent a class of models which are not linear at low doses. The multistage reduces to the one-hit if only two dose groups exist. With only two dose groups, the Weibull parameter k is set to 1 and the Weibull model also reduces to the one-hit model. Currently, as discussed previously, there is insufficient evidence to provide strong support for any low-dose extrapolation model, although there is some justification for low-dose linearity.

TABLE 3

Low-dose Extrapolation Models Used in Unit Risk Calculation

Model	Functional Form*	Comments
Multistage	$P(d) = 1-\exp(-q_o - q_1d^2-\ldots-q_kd^k)$	$q_i \geq 0$, all i
One-hit	$P(d) = 1-\exp(-q_o - q_1d)$	$q_o, q_1 \geq 0$
Weibull	$P(d) = 1-\exp(-q_o - q_1d^k)$	$q_o, q_1 \geq 0$
Log-probit	$P(d) = C + (1-C)\ \phi\ (A + B \log d)$	$\phi(x)$ = cumulative normal distribution ($\mu = o$, $\sigma^2 = 1$)
Time-to-Tumor	$P(d) = 1-\exp[(-q_o - q_1d - q_2d^2)(t-t_o)^k]$,	$t \geq t_o$, $q_i \geq 0$, all i, $k \geq 0$
	$= 0$	$t < t_o$, t_o = minimal induction time

*d = animal dose (mg/day). The parameters q_0, q_1, q_2 etc., A, B, C, t_o, k are all determined by statistically fitting each model to the animal dose-response data. P(d) = cancer risk at dose d. Note that unit risk is based on excess risk = (P(d)-P(0))/(1-P(0)), where P(0) is background incidence.

In addition, since the NCI (1976a,b, 1977) data provide information pertaining to early mortality and time-to-tumor estimates, a multistage Weibull model with five parameters (Crump, 1982) is also used (see Table 3). The time-to-tumor estimates are based largely on time-to-mortality with subsequent discovery of tumors. Since the NCI (1976a,b, 1977) studies are the only ones involving more than two dose groups, they are also the only data sets to which all four previous models can be applied.

Unit Risk Calculation Approach

Each low-dose extrapolation model is applied to the original unadjusted animal data. The resulting risk estimate is then converted into equivalent human unit risk by multiplication by several factors to adjust for experiment duration (if partial lifetime), species differences and, if necessary, route conversion. Uncertainties in each adjustment factor are investigated by changing the choice of each adjustment model and/or the assumed parameter values in the model. There is insufficient information to allow the alternatives to be characterized by likelihood or error distributions; hence, all decisions are based on scientific judgment. The adjustment categories and their decision alternatives are given in Table 4.

Each animal risk estimate obtained by fitting a low-dose extrapolation model to the animal data is presented as the maximum likelihood estimate (MLE) and, when possible, as the upper 95% confidence limit. The confidence limit is statistically more stable; the MLE may show substantial sensitivity to small changes in the original data. Some data errors are expected due to diagnostic uncertainties generally leading to underdetection, i.e., missed tumors. The confidence limit on risk, however, reflects sample size and random variability, and may be much higher than the MLE. Both estimates are investigated for their sensitivities to such data errors.

TABLE 4

Alternatives for Judgmental Decisions in Cancer Unit Risk Estimation

Decision Category	Alternatives
Low-dose extrapolation	Model: Multistage, one-hit, Weibull, log probit Estimator: Maximum likelihood estimate, upper 95% confidence limit
Equitoxic dose across species	Model: Dose/bw, dose/body surface area
Adjustment for partial lifetime experiment	Model: $(L/L_e)^k$, $k = 1\text{-}4$

The risk estimate from the low-dose extrapolation is based on average daily intake level. Gavage studies add uncertainty since gastrointestinal effects may be due to the repeated high local concentrations and may not occur if the same daily dose were given continuously, hence at a lower local concentration. Since insufficient pharmacokinetic information exists to adjust for intermittent exposure, the TWA daily intake rate is used.

The extrapolation from risk based on partial lifetime experiments to risk from lifetime exposure uses the factor: $(L/L_e)^k$, where L is lifespan and L_e is the duration of the experiment. As discussed previously, there is some evidence from human and animal studies to support an exponent of 3. Druckrey (1967) found that, at constant risk, fitting the model dt^n = constant to a series of data on nitrosamines gave values of n of 2-4. Studies of age-specific cancer rates in humans give estimates of k at 3 or higher. The concept of total dose (dose rate x duration) or similarly time-weighted-average dose for the experiment has been used as an indication of toxic severity, but has not been verified for cancer risk. It is included here for completeness and is represented by the case k=1 in the adjustment factor. There is insufficient information at present to allow precise determination of k for most chemicals. In the absence of such information, the sensitivity of the risk estimate to k is demonstrated by varying k between 1 and 4, and displaying the resulting range of risk estimates.

The conversion factor for species differences is presently based on models for equitoxic dose. The two general models currently used are based on body burden, dose/bw = constant (Stara and Kello, 1974); and metabolic rate, dose/body surface area = constant (Mantel and Schneiderman, 1975; Calabrese, 1982). In the absence of pharmacokinetic data related to toxicity on the test chemical for the experimental species and for humans, both models are used.

The conversion of administered dose from one route to another (e.g., ingestion to inhalation) is not well understood. The approach by Stockinger and Woodward (1958) uses approximate net absorption fractions and daily intake rate to convert from one route to an equitoxic exposure level via another route. This approach, discussed below, is used to give approximate estimates of exposure levels when insufficient data exist for the desired route, although the estimates are acknowledged as being highly uncertain and probably inaccurate.

Unit Risk Estimates for Ingestion Exposure

The unit risk estimates based on human equivalent doses consistent with previous guidelines (Federal Register, 1980b) are given in Table 5 for the four data sets and the applicable models. Note that for the NCI mouse and rat data, the unconstrained Weibull and unconstrained log probit models could not be used to estimate risk. The failure of the computer algorithm to yield meaningful results is attributed to the virtually flat slope at the low and high dose data. This caused the extrapolated risk to be essentially dose-independent, i.e., the same as the risk in the dosed groups regardless of the level of dose. The successfully applied models were fitted to the original animal data using average daily intake (mg/day); their parameter estimates are given in Table 6. The human risk was then calculated by multiplying the animal unit risk by the adjustment coefficients reflecting partial lifetime exposure and species conversion. The low-dose extrapolation of the NCI rat data is shown in Figure 1 as an example of the difference in unit risk estimation (risk at 1 μg/ℓ) due only to selection of extrapolation model. The risk projections have not been adjusted for species

TABLE 5

Unit Risk Estimates for Ingestion[a]

Study	Model (type of estimate)[b]					
	Multistage[c]		Time-to-Tumor		Weibull	Log Probit
	(MLE)	(UL)	(MLE)	(UL)		
Della Porta et al. (1961)	2.1E-5	3.4E-5	d/		e/	e/
Edwards et al. (1942)	7.1E-6	9.4E-6	d/		e/	e/
NCI (1976) mouse	1.4E-6	1.8E-6	1.8E-6	2.2E-6	f/	f/
NCI (1976) rat	1.9E-7	3.1E-7	3.1E-7	5.3E-7	f/	f/

[a] For ingestion of 1 μg CCl_4 per ℓ water daily for life. Species conversion uses dose/body surface area.

[b] MLE = Maximum likelihood estimate; UL = upper 95% confidence limit.

[c] The one-hit model results agreed with the multistage model results to three significant figures.

[d] No time-to-tumor data were available for these studies.

[e] These models have three parameters which could not be fit by the two dose groups in these studies.

[f] The unconstrained Weibull and unconstrained log probit models could not be used to estimate unit risk for these studies. See text.

TABLE 6

Parameter Estimates for the Low Dose Extrapolation Models Fitted to Animal Data[a]

Study	Multistage				Time-to-tumor				
	q_0	q_1	q_1^*	q_2	q_0	q_1	q_1^*	t_0	k
Della Porta et al., 1961	0	0.784	1.28	--	--	--	--	--	--
Edwards et al., 1942	0.0133	0.0415	0.0549	--	--	--	--	--	--
NCI, 1976 mouse	0.0397	0.133	0.167	0	0.13E-12	4.96E-13	5.90E-13	0	5.87
NCI, 1976 rat	0	2.71E-3	4.47E-3	0[b]	0	2.47E-13	4.15E-13	0	5.03

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[a] See Table 3 for model definitions. q_0 is dimensionless. q_1 and q_1^* are in $(mg/day)^{-1}$. q_2 is in $(mg/day)^{-2}$. t_0 is in (wk). All values are maximum likelihood estimates except q_1^*, which is the upper 95% confidence limit for q_1. E denotes powers of 10, i.e., 0.16E-13 = 0.16×10^{-13}.

[b] Estimates of q_3 and q_4 were also zero.

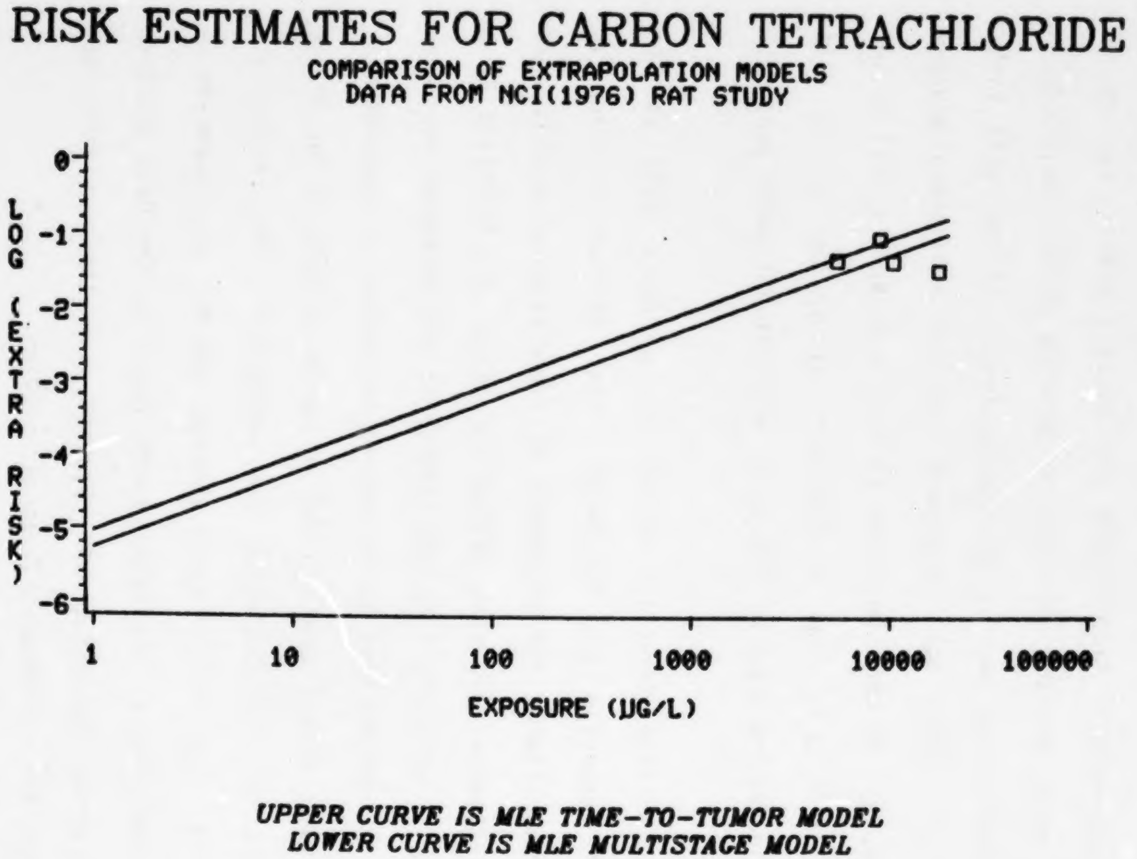

FIGURE 1

Comparison of low-dose extrapolation models. The models are applied to the NCI (1976) rat data: upper curve is MLE for time-to-tumor model, lower curve is MLE for multistage model. Risks are for animals. The curves are spline fits to the data; one data point not pictured is at the origin (0, 0). At 1 μg/ℓ the risk estimates from the curves differ by a factor of 17.

differences so that the actual rat response rates (the 4 data points) can be plotted. The logarithmic plot precluded the display of the control group response (0%).

The possibility of errors in the incidence data increase the uncertainty in the unit risk estimates. Such errors are usually under-detection of tumors (Anonymous, 1983), causing positively responding animals to be classified as nonresponders, and leading to underestimates of the unit risk. Furthermore, previous studies have suggested that the multistage maximum likelihood estimate, q_1, is more sensitive to such data errors than is the upper confidence limit, q_1*. The sensitivities of q_1 and q_1* to data changes are investigated for each of the three oral studies under consideration.

Discussion of possible detection errors in incidence data at the "ED_{01}" conference* suggested the following: that misclassification is more likely to underestimate the incidence of tumor-bearing animals than overestimate the incidence, and that having one animal misclassified in a study of 50 animals is not rare. The sensitivity of the parameter estimates to data errors was determined here by increasing the number of responders by 1 for every 50 animals tested (e.g., 1 for up to 50 animals, 2 for 51-100 animals, etc.), and then recalculating the parameters. The results are presented in Table 7. For each of these studies, the MLE (q_1) seems to be approximately as sensitive as the upper bound (q_1*) to the data changes. Note that the parameter estimates are obtained from the raw data, are unadjusted and, thus, are not comparable across studies.

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*"Workshop on Biological and Statistical Implications of the ED_{01} Study and Related Data Bases," Deer Creek State Park, Ohio, September 13-16, 1981.

TABLE 7

Sensitivity of Multistage Parameter Estimates to Changes in the Incidence Data

Study	Range of Parameter Values[a]			
	q_1 (MLE)	Relative Range[b]	q_1^* (upper limit)	Relative Range[b]
Della Porta et al., 1961	0.758-0.907	16%	1.26-1.46	14%
Edwards et al., 1942	0.0402-0.0452	11%	0.0537-0.0592	9%
NCI, 1976 (mouse)	0.151-0.152	0.6%	0.227-0.228	0.4%
NCI, 1976 (rat	1.45E-4, 1.23E-3	89%	1.02E-3, 1.95E-3	48%

[a] These estimates are unadjusted for species or duration and are not comparable across studies. Relative ranges are comparable across studies.

[b] Relative range = (high-low)/high. The original data do not necessarily give the lower estimate, so the relative range does not necessarily represent the change from the original parameter estimates.

In Table 5, in line with previous guidelines (Federal Register, 1980b), the species conversion model was dose per body surface area and partial lifetime risk was adjusted to lifetime risk by the ratio $(L/L_e)^3$. The effects of using dose per body weight instead for species conversion, as well as exponents of 1 (total dose), 2 or 4 for the partial lifetime to lifetime adjustment are shown by the coefficients in Table 8 and the range of unit risk estimates in Table 9. Note that the adjustment coefficients in Table 8 are comparable only within a study, not across studies. The unit risk estimates in Table 9 are comparable across studies.

UNIT RISK ESTIMATES FOR INHALATION EXPOSURE

The unit risk for inhalation exposure is the excess cancer risk for lifetime exposure to 1 µg CCl_4/m^3 air. No inhalation cancer studies have been located which contain adequate dose-response information. However, the unit risk can be estimated from ingestion studies by assuming that the same daily intake rate results in the same lifetime risk. This assumption has not been thoroughly tested with other chemicals. In addition, each of the studies used for estimating oral unit risk has deficiencies. Therefore, the unit risk estimate for inhalation exposure should be considered approximate based on assumptions that have yet to be proven.

To estimate the risk corresponding to the concentration of 1 µg CCl_4/m^3 air, the effective dose in terms of mg/kg/day corresponding to 1 µg/m³ must first be estimated. Assuming an air intake of 20 m³/day (ICRP, 1975) and a 40% absorption rate by inhalation for humans (as recommended in this document), this effective dose can be estimated for a 70 kg human:

$$d_{air} = [20\ m^3/day \times 0.40 \times 1/(70\ kg)] \times 10^{-3}\ mg/m^3$$

$$= 1.14 \times 10^{-4}\ mg/kg/day = 7.98 \times 10^{-3}\ mg/day$$

TABLE 8

Adjustment Coefficients for Estimating Human Unit Risk (duration adjustment) X (species adjustment)

Species Conversion: duration exponent k		Study[a]			
		Della Porta et al. (1961)	Edwards et al. (1942)	NCI (1976a,b, 1977) (mouse)	NCI (1976a,b, 1977) (rat)
Surface area:	1	0.0134	0.0140	0.00543	0.0346
	2	0.0134	0.0345	0.00543	0.0346
	3[b]	0.0134	0.0852	0.00543	0.0346
	4	0.0134	0.210	0.00543	0.0346
Body weight:	1	0.00154	0.00105	0.00040	0.00643
	2	0.00154	0.00259	0.00040	0.00643
	3	0.00154	0.00640	0.00040	0.00643
	4	0.00154	0.0158	0.00040	0.00643

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[a] Only the Edwards et al. (1942) study had partial lifetime experiment duration requiring the duration adjustment factor: (fraction lifespan)$^{-k}$.

[b] The animal unit risk estimates were multiplied by the coefficients in this row (k = 3 in duration factor, dose/surface area species conversion) to obtain the human unit risk estimates in Table 4.

TABLE 9

Range of Unit Risk Estimates for Oral Exposure[a]

Study	Multistage Model		Time-to-Tumor Model	
	MLE	Upper Limit	MLE	Upper Limit
Della Porta et al. (1961)	2.4E-6, 2.1E-5	3.9E-6, 3.4E-5	NA[b]	
Edwards et al. (1942)	8.7E-8, 1.7E-5	1.2E-7, 2.3E-5	NA	
NCI (1976) mouse	1.0E-7, 1.4E-6	1.3E-7, 1.8E-6	1.3E-7, 1.8E-6	1.6E-7, 2.2E-6
NCI (1976) rat	3.5E-8, 1.9E-7	5.8E-8, 3.1E-7	5.8E-8, 3.1E-7	9.8E-8, 5.3E-7

[a] Obtained by changing the adjustment coefficients for species differences and partial lifetime experiment duration. See text.

[b] NA = Not applicable. Time-to-tumor data not available.

which is 4.0 times the unit intake for oral exposure (1 μg/ℓ = 2×10^{-3} mg/day). The unit risk for inhalation is then estimated from the oral studies by multiplying the oral unit risk by 4.0. Based on the range of recommended risks (based upon present information and current understanding of carcinogenesis) derived from the four oral data sets, and using the linearized multistage model with the dose per body surface area conversion across species, and with the exponent k=3 in the adjustment for partial lifetime study, the upper limit estimate of unit risk for inhalation exposure ranges from 1.2×10^{-6} to 1.4×10^{-4} with a geometric mean of 1.5×10^{-5}. As a measure of uncertainty, all models and adjustments were considered (see Table 9). The resulting upper limit unit risk estimate for inhalation exposure ranged from 2.3×10^{-7} to 1.4×10^{-4}.

Because of the uncertainties in both the qualitative and quantitative aspects of risk assessment, the actual cancer risks may be lower than the best unit risks presented above, which should be regarded only as plausible upper-limits. The unit risk estimates are calculated using a dose-response extrapolation model which is linear at low doses. This low-dose linearity is based on mutagenicity studies and on some similarities between mutagenesis and carcinogenesis. Since the results on the mutagenicity of CCl_4 are inconclusive, the selected extrapolation model may be inappropriate, and hence the unit risk estimates are uncertain.

MULTIPLE EXPOSURE SITUATIONS

The above information provides recommended route-specific cancer risk estimates associated with exposure to given units of CCl_4. These estimates may be conservative due to the choice of the multistage model for dose extrapolation and the various adjustment factors. Nevertheless, unit risks for cancer presented above are defined for independent water and air exposures in that their computation assumes 100% of the insult is via the stated route.

When exposure is by both oral and inhalation routes, an additivity assumption can be used to calculate the risk associated with the concurrent exposures. It is a general recommendation to use the additivity assumption which is made since the available data on CCl_4 are limited and do not allow the presentation of a defensible alternative. As new information becomes available, other alternatives should be considered. Here the additivity assumption is that the risk associated with exposure to a given chemical via two routes concurrently is roughly the sum of the risks associated with each independent route-specific exposure. Since interactions between the concurrent routes of intake cannot be quantified, uncertainty surrounds the resulting risk estimate that is derived from the concurrent risks.

In applying the assumption of additivity, the risks rather than the doses associated with each route are added, but the mere summation of these risks is presently justifiable only when doses are low enough that no interaction occurs between the two routes. Furthermore, the amounts of 1 µg/ℓ and 1 µg/m³ are concentrations in water and air, respectively, not doses. The dose can be estimated by assuming consumption of 2 ℓ water/day over the lifetime. Thus, the daily dose corresponding to a concentration of 1 µg/ℓ water would be 2 ℓ/day x 1 µg/ℓ = 2 µg/day.

COMPARISON OF RISK ESTIMATES FOR VARIOUS CARCINOGENS

The carcinogenic risk from exposure to CCl_4 is compared to the risk from exposure to other potential carcinogens in Table 10. For comparison

TABLE 10

Relative Carcinogenic Potencies Among 53 Chemicals Evaluated by the Carcinogen Assessment Group as Suspect Human Carcinogens[a,b,c]

Compounds	Slope $(mg/kg/day)^{-1}$	Molecular Weight	Potency Index	Order of Magnitude (log_{10} index)
Acrylonitrile	0.24 (W)	53.1	$1x10^{+1}$	+1
Aflatoxin B_1	2924	312.3	$9x10^{+5}$	6
Aldrin	11.4	369.4	$4x10^{+3}$	+4
Allyl chloride	$1.19x10^{-2}$	76.5	$9x10^{-1}$	0
Arsenic	15 (H)	149.8	$2x10^{+3}$	+3
B[a]P	11.5	252.3	$3x10^{+3}$	+3
Benzene	$5.2x10^{-2}$ (W)	78	$4x10^{0}$	+1
Benzidine	234 (W)	184.2	$4x10^{+4}$	+5
Beryllium	1.40 (W)	9	$1x10^{+1}$	+2
Cadmium	6.65 (W)	112.4	$7x10^{+2}$	+3
Carbon tetrachloride	0.13	153.8	$2x10^{+1}$	+1
Chlordane	1.61	409.8	$7x10^{+2}$	+3
Chlorinated Ethanes				
1,2-dichloroethane	$6.9x10^{-2}$	98.9	$7x10^{0}$	+1
1,1,2-trichloroethane	$5.73x10^{-2}$	133.4	$8x10^{0}$	+1
1,1,2,2-tetrachloroethane	0.20	167.9	$3x10^{+1}$	+1
Hexachloroethane	$1.42x10^{-2}$	236.7	$3x10^{0}$	0
1,1,1-trichloroethane	$1.6x10^{-3}$	133.4	$2x10^{-1}$	-1
Chloroform	$7x10^{-2}$	119.4	$8x10^{0}$	+1

TABLE 10 (cont.)

Compounds	Slope (mg/kg/day)-1	Molecular Weight	Potency Index	Order of Magnitude ($\log_{10}$ index)
Chromium	41 (W)	104	$4x10^{+3}$	+4
DDT	8.42	354.5	$3x10^{+3}$	+3
Dichlorobenzidine	1.69	253.1	$4x10^{+2}$	+3
1,1-Dichloroethylene	0.147 (I)	97	$1x10^{+1}$	+1
Dieldrin	30.4	380.9	$1x10^{+4}$	+4
Dinitrotoluene	0.31	182	$6x10^{+1}$	+2
Diphenylhydrazine	0.77	180	$1x10^{+2}$	+2
Epichlorohydrin	$9.9x10^{-3}$	92.5	$9x10^{-1}$	0
Bis(2-chloroethyl)ether	1.14	143	$2x10^{+2}$	+2
Bis(chloromethyl)ether	9300 (I)	115	$1x10^{+6}$	+6
Ethylene dibromide (EDB)	8.51	187.9	$2x10^{+3}$	+3
Ethylene oxide	1.26 (I)	44.1	$6x10^{+1}$	+2
Heptachlor	3.37	373.3	$1x10^{+3}$	+3
Hexachlorobenzene	1.67	284.4	$5x10^{+2}$	+3
Hexachlorobutadiene	$7.75x10^{-2}$	261	$2x10^{+1}$	+1
Hexachlorocyclohexane				
technical grade	4.75	290.9	$1x10^{+3}$	+3
alpha isomer	11.12	290.9	$3x10^{+3}$	+3
beta isomer	1.84	290.9	$5x10^{+2}$	+3
gamma isomer	1.33	290.9	$4x10^{+2}$	+3

TABLE 10 (cont.)

Compounds	Slope (mg/kg/day)$^{-1}$	Molecular Weight	Potency Index	Order of Magnitude (log$_{10}$ index)
Methylene chloride	6.3×10^{-4}	84.9	5×10^{-2}	-1
Nickel	1.15 (W)	58.7	$7\times10^{+1}$	+2
Nitrosamines				
Dimethylnitrosamine	25.9 (not by q_1*)	74.1	$2\times10^{+3}$	+3
Diethylnitrosamine	43.5 (not by q_1*)	102.1	$4\times10^{+3}$	+4
Dibutylnitrosamine	5.43	158.2	$9\times10^{+2}$	+3
N-nitrosopyrrolidine	2.13	100.2	$2\times10^{+2}$	+2
N-nitroso-N-ethylurea	32.9	117.1	$4\times10^{+3}$	+4
N-nitroso-N-methylurea	302.6	103.1	$3\times10^{+4}$	+4
N-nitroso-diphenylamine	4.92×10^{-3}	198	1×10^{0}	0
PCBs	4.34	324	$1\times10^{+3}$	+3
Phenols				
2,4,6-trichlorophenol	1.99×10^{-2}	197.4	4×10^{0}	+1

TABLE 10 (cont.)

Compounds	Slope $(mg/kg/day)^{-1}$	Molecular Weight	Potency Index	Order of Magnitude (log_{10} index)
Tetrachlorodibenzo-p-dioxin (TCDD)	$1.56x10^5$	322	$5x10^{+7}$	+8
Tetrachloroethylene	$3.5x10^{-2}$	165.8	$6x10^0$	+1
Toxaphene	1.13	414	$5x10^{+2}$	+3
Trichloroethylene	$1.9x10^{-2}$	131.4	$2.5x10^0$	0
Vinyl chloride	$1.75x10^{-2}$ (I)	62.5	$1x10^0$	0
Vinylidene chloride	0.13 (I)	97	$1x10^{+1}$	+1

[a] Animal slopes are 95% upper-limit slopes based on the linear multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure, and H (human drinking water exposure). Human slopes are point estimates, based on linear non-threshold model.

[b] The potency index is a rounded-off slope in $(mMol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

[c] Not all the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

purposes, the risks are based on lifetime esposure to 1 mg/kg/day. These estimates are derived from various studies on humans and animals, for oral and inhalation exposure. These estimates are derived with the linearized multistage model with species adjustment using dose/body surface area and adjustment for partial lifetime study by using the exponent k=3. Carbon tetrachloride has a relatively low potency compared to the others in the group.

A relative potency index, proposed by the Carcinogen Assessment Group, is also presented in Table 10 for each chemical. This index represents the risk posed by daily exposure to 1 mMol of carcinogen per kg body weight, and thus allows comparison of risks from exposure to the same number of molecules. The frequency distribution of the relative potency indices, rounded to the nearest order of magnitude, is shown in Figure 2.

SUMMARY/CONCLUSIONS

No single study was entirely adequate for risk estimation. Thus, the unit risk estimate is based on the geometric mean of the individual unit risk estimates from the four studies considered. The studies contained data on three animal species: rats, mice and hamsters. From these data, the recommended upper limit unit risk estimates (based upon present information and current understanding of carcinogenesis) for ingestion are in the range of 3.1×10^{-7} to 3.4×10^{-5} with a geometric mean of 3.7×10^{-6}. Using these same oral data, unit risk estimates for inhalation are in the range of 1.2×10^{-6} to 1.4×10^{-4} with a geometric mean of 1.5×10^{-5}. Since no study was entirely adequate for risk assessment purposes, the geometric mean of the upper confidence limits is preferred as the most plausible upper limit estimate of unit risk.

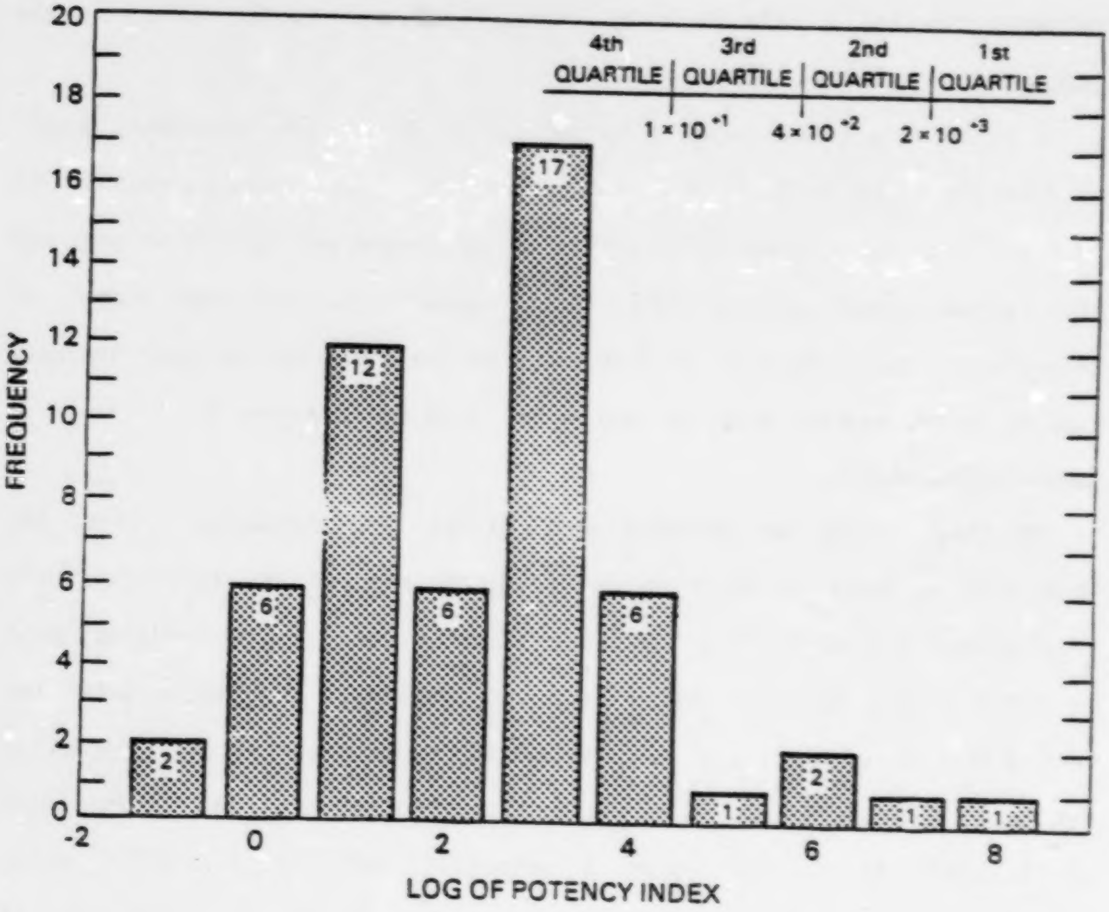

FIGURE 2

Histogram Representing the Frequency Distribution of the Potency Indices of 54 Suspect Carcinogens Evaluated by the Carcinogen Assessment Group